Gene Expression in *Streptococcus Mutans* Biofilms

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Summary

Streptococcus mutans is considered the major aetiological agent of human dental caries. It is an obligate biofilm-forming bacterium, which resides on teeth and forms, together with other species, an oral biofilm that is often designated as supragingival plaque.

This thesis consists of three distinct parts. The first part describes, using microarray analysis, how *S. mutans* modulates gene expression when grown under different conditions in biofilms. The goal of this analysis was to identify genes that are required for establishment and survival in biofilms. When grown in exponential biofilms *S. mutans* increased the expression of uptake systems for Mn^{2+}, suggesting a requirement for this element for proper establishment in biofilm. Furthermore, *S. mutans* down-regulated genes associated with sucrose-dependent adhesion (*spaP, gtfB*). When exponential biofilms were cultivated in the presence of sucrose, a specific sucrose-transporter was activated.

Compared with planktonic growth, stationary phase biofilms showed a greater change in their gene expression profile than biofilms harvested from the exponential growth phase, indicating that different regulation modes of growth processes are operating under altered environmental conditions.

Again compared with planktonic growth, stationary phase biofilms grown with glucose showed up-regulation of genes involved in carbohydrate metabolism and signal transduction, and down-regulation of genes associated with translation, energy production and amino-acid transport, and metabolism. A gene cluster comprising the *lrgA* and *lrgB* genes, which are predicted to be involved in cell death, was also highly up-regulated in these biofilms.

Comparison of the transcriptome of a sucrose-grown stationary biofilm with that of a glucose-grown stationary biofilm culture suggested that there may be more DNA damage in the former, since several genes coding for repair enzymes were up-regulated. Energy consumption and the metabolism of amino-acids and sugars were decreased.

When comparing starved biofilms with fed biofilms *of S. mutans*, the latter showed an increase in expression of genes required for transcription and translation, as well as of genes incoding a specific PTS system responsible for the uptake of sucrose. The opposite pattern was observed when biofilms were transfered from replete medium to starving conditions.

In the second part of this work, the involvement of the two gene clusters *lrgAB* and *cidAB* in cell death was investigated. Using real-time RT-PCR, it was found that expression of *lrgAB* increased with rising cell density. A similar pattern was observed for *lytSR*, which encodes a two-component

signal transduction system. Further analysis suggested that the *lrgAB* genes were negatively regulated by LytSR. The *lrgAB* genes appear to be important for long-term survival, since a *lrgAB* knock-out mutant exhibited a $3\log_{10}$-decrease in viable cell count after 13 days of incubation. The expression of *lrgAB* increased in response to lactic acid, the predominant organic acid found in culture media from bacteria grown with sucrose as the sole carbon source. The exposure of *S. mutans* to CCCP, an ionophore that affects both components of the proton motive force, resulted in high expression of *lrgAB*. Biofilm formation was not affected in both *lrgAB* and *cidAB* mutants. Overall, it was shown that the *lrgAB* gene cluster encodes an important factor for *S. mutans* survival in stationary phase. This factor is probably required to withstand low pH, starvation, or membrane-damaging agents.

Bacteria can detect, transmit and react to signals from the outside world by two-component systems and by serine-threonine kinases and phosphatases first detected eukaryotic cells, but later recognized to occur widespread in bacteria as well. *S. mutans* contains one such serine-threonine kinase, encoded by *pknB*. A gene encoding serine-threonine phosphatase, *pppL*, is located upstream of *pknB*.

In the third part of the work, the phenotypes of single mutants in *pknB* and *pppL* and of a *pknB* - *pppL* double mutant were characterized. All mutants showed abnormal cell shapes and grew slower than the wild type. Whole genome transcriptome analysis revealed that a *pknB* mutant showed reduced expression of genes in bacteriocin production and genetic competence. Among the genes that were differentially regulated in the *pknB* mutant several were likely involved in cell-wall metabolism. One such gene, SMU.2146c, and two genes encoding bacteriocins were shown to be also down-regulated in a mutant in vicK, a sensor kinase involved in response to oxidative stress. Collectively, the results indicate that PknB can modulate the activity of the two-component signal transduction systems VicKR and ComDE.

Zusammenfassung

Streptococcus mutans gilt als wichtigster Verursacher der humanen Karies. *S. mutans* ist ein obligat Biofilm bildendes Bakterium, das auf der Oberfläche von Zähnen wächst und zusammen mit anderen Bakterien einen oralen Biofilm bildet, der allgemein als supragingivale oder auch koronale Plaque bezeichnet wird.

Diese Dissertation besteht aus drei Teilen. Der erste Teil beschreibt wie *S. mutans* Bakterien ihre Genexpression ändern, wenn sie unter verschiedenen Bedingungen im Biofilm wachsen. Das Ziel dieser mit Microarrays ausgeführten Analyse war es, Gene, welche für die Etablierung und das Überleben im Biofilm notwendig sind, zu identifizieren. Im Vergleich zu planktonischen Kulturen erhöhte *S. mutans* in exponentiell wachsenden Biofilmen die Expression von Genen, die für die Mn^{2+}-Aufnahme kodieren. Dies weist auf einem erhöhten Bedarf an Mn^{2+} Ionen bei der Biofilm Bildung hin. Weiter wurden mit der Saccharose-abhängigen Adhäsion assoziierte Gene in ihrer Expression herabreguliert (*spaP*, *gtfB*). Umgekehrt erwies sich in in Anwesenheit von Saccharose gezüchteten und aus der exponentiellen Wachstumsphase geernteten Biofilmen, das für einen spezifischen Saccharose-Transporter kodierende Gen als aufreguliert.

Im Vergleich mit planktonisch wachsenden Zellen wiesen Biofilme in der stationären Phase eine grössere Änderung im Transkriptom auf als Biofilme in der exponentiellen Wachstumsphase, was auf unterschiedliche Formen der Regulation der Wachstumsprozesse hinweist. Gene deren Produkte am Zuckermetabolismus und in der Signaltransduktion beteiligt sind waren in Biofilmen aus der stationären überexprimiert und Gene welche mit Translation, Energieproduktion und Aminosäuretransport und Aminosäuremetabolismus assoziiert sind, erwiesen sich als herunterreguliert. Der *lrgAB* Genkomplex, dessen Produkt wahrscheinlich am Zelltod beteiligt ist, war in Biofilmen stark aufreguliert.

Wenn Saccharose statt Glukose als Kohlehydratquelle im Medium angeboten wurde, führte dies in stationären Biofilmen zur Aufregulierung von Genen welche für DNA Reparaturenzyme kodieren. Dies deutet darauf hin, dass es in Saccharose-gewachsenen Biofilmen vermehrt zu DNA-Schädigungen kommt. Die Transkription von Genen, welche am Energieverbrauch sowie am Metabolismus von Aminosäuren und Zuckern beteiligt sind, wurde in mit Saccharose gezüchteten, stationären Biofilmen herunterreguliert.

Der Wechsel von Biofilmen aus Speichel in ein nährstoffreiches Medium führte zu einer

Zunahme der Expression von Genen mit Bedeutung in der Kontrolle von Transkription und Translation. Ebenso wurde das Gen kodierend für ein Saccharose-PTS-System markant aufgeguliert. Das Gegenteil wurde nach der Verlagerung von Biofilmen von einem nährstoffreichen Medium in Speichel beobachtet.

Im zweiten Teil der Arbeit wurde die Beteiligung der beiden Genkomplexe *lrgAB* und *cidAB* am Zelltod untersucht. Mittels Real-Time RT-PCR konnte beobachtet werden, dass die Expression von *lrgAB* durch das Zwei-Komponenten-Signaltransduktion-System LytSR negativ reguliert wurde. Die Expression von *lrgAB* und *lytSR* nahm mit zunehmender Zelldichte zu. Die *lrgAB* Gene sind vermutlich für das langfristige Überleben von *S. mutans* Zellen wichtig, da eine *lrgAB* Knock-out-Mutante im Vergleich zum Wildtyp Stamm nach 13 Inkubation Tagen eine Abnahme der Anzahl an lebensfähigen Zellen um 3 log-Stufen aufwies. Die Anwesenheit im Medium von Milchsäure – die prädominant produzierte organische Säure wenn *S. mutans* mit Saccharose als Kohlenstoffquelle wächst – führte zu einem Anstieg der *lrgAB* Expression. Ebenfalls zu höherer Expression von *lrgAB* führte die Exposition von *S. mutans* gegenüber CCCP, einem Ionophor das beide Komponenten des Systems inhibiert, welches den Zellwand Protonengradienten steuert. Ein Unterschied in der Biofilm-Bildung konnte aber weder für *lrgAB* noch für *cidAB* Mutanten nachgewiesen werden. Insgesamt zeigen die Befunde, dass die Gene des *lrgAB* Komplexes für einen Faktor kodieren, welcher für das Überleben von *S. mutans* in der stationären Phase wichtig ist. Dieser Faktor ist wahrscheinlich notwendig um bei niedrigen pH-Werten, bei Mangel an Nährstoffen oder bei Vorliegen von Membranschäden überleben zu können.

Bakterien können Signale aus der Außenwelt mit Zweikomponenten-Signaltransduktionssystemen und mit Hilfe von Serin/Threonin Kinasen, sowie den dazugehörenden Phosphatasen, übertragen. *S. mutans* enthält eine Serin/Threonin Kinase, die durch *pknB* kodiert wird. Das *pppL* Gen, welches eine Serin/Threonin Phosphatase kodiert, befindet sich stromaufwärts von *pknB*.

Im dritten Teil der Arbeit wurden die Phänotypen von Mutanten der *pknB* und *pppL* Gene untersucht. Die Mutanten zeigten abnormale Zellformen und wuchsen im Vergleich zum Wild-Typ Stamm langsamer. Die Transkriptom Analyse zeigte, dass in der *pknB* Mutante die Expression von Genen herabreguliert war, welche für Bakteriozine kodieren. Ebenso betroffen waren Gene mit Bedeutung in der Steuerung genetischer Kompetenz und des Zellwandmetabolismus. Ein solches Metabolismusgen (SMU.2146c) und zwei Bakteriozingene erwiesen sich auch in einer *vicK* Mutante,

welche für eine Sensor-Kinase kodiert, als herabreguliert. Insgesamt zeigen diese Ergebnisse, dass PknB die Aktivität der Zweikomponenten Signaltransduktionssysteme VicKR und ComDE modulieren kann.

I. INTRODUCTION

1.1 Genus *Streptococcus* and *Streptococcus mutans*

Streptococcus is a genus of Gram-positive bacteria belonging to the Phylum Firmicutes. In these non-motile bacteria, cellular division occurs along a single axis and therefore, they grow in chains or pairs. Streptococci are catalase- and oxidase-negative, and many are facultative anaerobes. They ferment carbohydrates to lactic acid and hence belong to the group of lactic acid bacteria. In particular β-hemolytic streptococci may cause severe infections of the upper respiratory tract, the skin and the subcutaneous connective tissue. The γ-haemolytic or non-haemolytic streptococci rarely cause disease. α-haemolytic streptococci (often called viridians streptococci) are part of the normal commensal flora of the skin and in particular of the mouth. In susceptible individuals streptococci of oral origin are important causative organisms of endocarditis.

Streptococcus mutans is a γ-haemolytic bacterium commonly found on the surface of teeth. It took its name from the different morphologies that it exhibited upon growth on different sugars. *S. mutans* was first isolated from carious lesions by J.K. Clarke in 1924 (Clarke, 1924). His description was as follows:

> *"S. mutans was isolated from 36 of the 50 teeth. Acid is very rapidly produced, the medium, originally pH 7, giving a reaction of pH 4.2 in about 24 hours. All the strains isolated ferment glucose, lactose, raffinose, mannite (mannitol), inulin, and salicin with production of acid. There is usually neither haemolysis nor discoloration on blood-agar. The fact that the colonies of S. mutans adhere closely to the surface of the teeth appears to be of great importance."*

Later in that decade, *S. mutans* was identified in a high percentage in dental caries, suggesting a possible role in tooth decay. It was not until the late 1950s that Orland (Orland 1959) and Fitzgerald (Fitzgerald & McDaniel, 1960) showed that inoculation of *S. mutans* in germ-free rats and hamsters led to formation of dental caries.

Currently, seven distinct species of human and animal mutans streptococci and eight serotypes (*a-h*) are recognized, based on the antigenic properties of their cell-wall carbohydrates. The species *Streptococcus mutans* comprises the three serotypes *c, e* and *f* (Samaranayake, 2002).

1.2 Dental caries

Dental caries is a disease manifested by the destruction of dental hard tissues by acidic

components produced by bacterial metabolism, especially by sugar fermentation. Dental caries is a wide-spread disease which, in an advanced form, leads to tooth decay. There are several host factors such as teeth and saliva which interact with acid-producing bacteria and contribute to the onset and progression of caries.

Carious demineralizations can be seen on the hard dental tissues, but the disease process is initiated within the bacterial biofilm (known also as dental plaque) that covers a tooth surface (Selwitz *et al.*, 2007). Carries can be divided in smooth surface carries and in pits and fissure caries. Smooth surface caries occurs with low frequency in industrialized countries because the teeth surfaces are readily accessible and therefore easy to clean. Pits and fissures are anatomical regions on a tooth where the enamel folds inward. For all types of pits and fissures, the incidence of dental caries is higher than for smooth surfaces, because of the difficulty in cleaning these surfaces. Pit and fissure caries represent 90% of all dental caries among children.

Dental carries may appear when the ecology of supragingival oral biofilms is disequilibrated (Fejerskov, 2004; Scheie & Petersen, 2004), resulting in preferential proliferation of several acid-producing bacterial species such as *S. mutans* and lactobacili at the expense of other bacterial inhabitants of the oral biofilms (Caufield *et al.*, 2007; Li *et al.*, 2007).

1.3 Virulence factors of *Streptococcus mutans*

In most infectious diseases with a highly specific bacterial aetiology, virulence factors, such as toxins, are responsible for the damage caused to the host. In the pathology of dental caries, damage to the teeth is associated almost exclusively with bacterial metabolism. Mutans streptococci are considered to be the most cariogenic organisms among the oral microbiota because they are both highly acidogenic and rather aciduric. Metabolism of the nutrients from saliva and the host's diet creates stressors such as acids, reactive oxygen species, and other agents that can cause damage to biomolecules. Thus, stress tolerance by the bacteria themselves is intimately connected with virulence (Lemos & Burne, 2008). The virulence of *S. mutans* can be associated with four main attributes: its ability to form biofilms on the tooth surface, to produce a sticky water-insoluble extracellular glucose-polymer, to produce large quantities of organic acids (acidogenicity) from metabolism of a wide range of carbohydrates, and to tolerate environmental stresses, particularly low pH (aciduricity).

1.3.1 Acidogenicity

A considered virulence factor of *S. mutans* is acidogenicity, the ability of bacteria to generate acidic end products that lower the pH of the environment. *S. mutans* is certainly one of the most acidogenic species found in dental plaque, because it is capable of producing acid from a wide range of fermentable carbohydrates at a higher rate and over a much greater pH range than most other streptococci (de Soet *et al.*, 2000).

1.3.2 Aciduricity

Tolerance of acidic conditions and an the ability to mount an adaptive response upon exposure to low pH are also of major importance in the pathogenesis of dental caries. *S. mutans* employs a number of strategies to resist the effects of acid stress, and earlier studies have highlighted the role of the proton-translocating ATPase (H^+-ATPase) as a key factor contributing to the aciduric response. Thus, this organism is a successful pathogen not only because it produces acids which demineralize the enamel but also because its aciduric characteristics (Dashper & Reynolds, 1992). The ability of the streptococci to cope with acidification in dental plaque has to do with the problem of survival in the presence of organic acids, which are secreted as anions and their conjugate protons. As the pH of plaque acidifies, protons rapidly diffuse across the bacterial membrane and begin to acidify the interior of the cell. The relatively acid-sensitive glycolytic enzymes begin to lose activity, severely affecting the cell's ability to produce ATP; and internal acid pH values begin to cause structural damage to molecules such as DNA and proteins (Quivey *et al.*, 2001). In *S. mutans*, the minimum pH value at which glycolysis can occur is 4.4, whereas the minimum pH at which growth can occur is 4.8 (Bender *et al.*, 1985), indicating that ATP can still be produced by the organism's ATPase under conditions that no longer allow its utilization for growth. The lower the pH at which the ATPase can function as the metabolic end-products build up, the more competitive the organism is in a biofilm environment. The central role of ATPase is also seen in enteric bacteria, where it has been shown that the adaptive acid-tolerance response does not occur in cells that are defective in the F-ATPase (Foster & Hall, 1991).

1.3.3 Bacteriocins and immunity proteins

Another potential virulence factor of mutans streptococci may be their production of bacteriocins. Bacteriocins (mutacins) are ribosomally synthesized peptides, which have antimicrobial activity against closely related bacteria (Sablon *et al.*, 2000). In *S. mutans*, two types of mutacins have been identified: lantibiotics (post-translationally modified bacteriocins), represented by mutacin I, II

and III and, non-lantibiotics (unmodified bacteriocins), represented by mutacin IV. The lantibiotics have a broad target spectrum including most of Gram-positive bacteria, whereas the non-lantibiotics have a narrower spectrum. They kill mostly streptococci belonging to *S. sanguinis* or other members of the mitis group (Qi *et al.*, 1999; Qi *et al.*, 2001).

The activity of bacteriocins can help *S. mutans* compete for limited nutrients available in its ecological niche. By concurring bacteriocins production with population density, *S. mutans* can not only reduce its competitors for food, but also ensure that high amounts of heterologous DNA are present during genetic competence development. In addition to producing bacteriocins, *S mutans* was shown to produce bacteriocin-immunity proteins, which can modulate the sensitivity to antimicrobials in *S. mutans* (Matsumoto-Nakano & Kuramitsu, 2006).

1.3.4 Carbohydrate uptake and metabolism in *S. mutans*

S. mutans is able to take up and metabolize a wide variety of sugars, which are almost exclusively used as carbon sources for generation of energy. In the natural environment of *S. mutans*, the oral cavity, carbohydrate concentrations are low. Therefore, the bacterium must reside on sugars coming from the dietary food. Since the medium is permanently changing, the bacterium must select the most suitable energy source and take up preferred sugars to be able to compete and survive in this dynamic niche. In *S. mutans*, carbohydrates are mainly taken up by 2 types of transporters: phosphoenolpyruvate-sugar phosphotransferase systems (PTSs) and ATP-binding cassette (ABC) transporters.

In PTS, phosphorylation and uptake of carbohydrates are coupled. A PTS is composed of two non-specific energy–coupling systems, designated Enzyme I (EI) and heat-resistant protein (HPr), and a sugar–specific, membrane-bound permease complex, called Enzyme II (EII). EII itself consists of three or four subunits: EIIA and EIIB are located in the cytoplasm whereas EIIC and in some cases EIID are located in the membrane, where they act as a channel (Vadeboncoeur & Pelletier, 1997) Each PTS uses phosphoenolpyruvate as phosphate donor to phosphorylate sugars via a phosphoryl–transfer system involving EI, HPr and EIIA (**Figure 1**). It is believed that bacteria use PTS for uptake at low sugar concentrations (in the µmolar range) (Ellwood *et al.*, 1979). Based on the genome sequence, 14 PTS have been identified in *S. mutans* (Ajdić *et al.*, 2002). The involvement of PTS in sugar uptake have been shown for glucose (Schachtele & Mayo, 1973), sucrose (Slee & Tanzer, 1979a; Slee & Tanzer, 1979b; St Martin & Wittenberger, 1979), mannose (Néron & Vadeboncoeur, 1987b), mannitol

and sorbitol (Brown & Wittenberger, 1973; Maryanski & Wittenberger, 1975), maltose (Würsch & Koellreutter, 1985), lactose (Calmes, 1978), fructose (Gauthier *et al.*, 1984), trehalose (Poy & Jacobson, 1990), xylitol (Trahan *et al.*, 1985) and *N*-acetylglucosamine (Jacobson *et al.*, 1990).

Both EI and HPr have been purified from *S. mutans* (Boyd *et al.*, 1994; Mimura *et al.*, 1984). EI and HPr are encoded by the *ptsI* and *ptsH* genes, respectively. Sugar-specific EIIA components such as $EIIA^{Fru}$ (fructose uptake) and $EIIA^{Lac}$ (lactose uptake), have also been purified from oral streptococci (Gauthier *et al.*, 1984; Vadeboncoeur & Proulx, 1984).

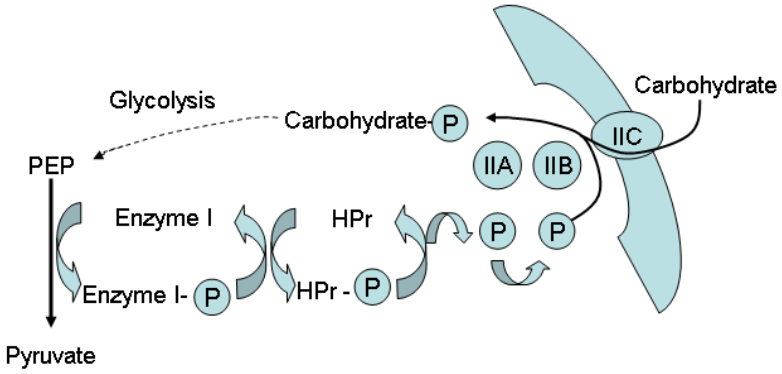

Figure 1. Schematic representation of the general components of the phosphoenolpyruvate:sugar phosphostransferase system (PTS): Enzyme I, HPr and Enzyme II with three subcomponents (kinases IIA and IIB and permease IIC). Modified after Vadeboncoeur & Pelletier, 1997.

There are at least 2 PTS in *S. mutans* that transport glucose (Néron & Vadeboncoeur, 1987a). It has been shown that one of them ($EII^{Man/Glu}$) is constitutively expressed and not only transports glucose, but also mannose and 2-deoxyglucose (Abranches *et al.*, 2003), whereas the second (EII^{Glu}) can transport glucose, 2-deoxyglucose and α-methylglucoside (Vadeboncoeur & Proulx, 1984). Ajdic & Pham (2007) showed that this PTS can transport also maltose and maltodextrin, renaming it ($EII^{Mal/Glu}$).

Several transporters can take up sucrose in *S. mutans*. Following growth in sucrose, the high affinity sucrose PTS EII^{Suc} is induced. The gene for EII^{Suc}, *scrA*, is located in an operon that contains also the *scrB* gene which encodes sucrose-6-phosphate hydrolase, converting sucrose-6-phosphate into glucose-6-phosphate and fructose. The trehalose PTS is also involved in the uptake of sucrose (Poy & Jacobson, 1990). Fructose activates a specific fructose PTS (EII^{Fru}), which converts fructose to fructose 1-phosphate. This is then further metabolized to 1-phosphofructokinase (Gauthier *et al.*, 1984). Furthermore, fructan polymers synthesized from sucrose by the action of fructosyltransferases to

produce extracellular storage compounds (Burne *et al.*, 1996) can be degraded again by the action of fructan hydrolases (fructanases) releasing fructose. Fructose can also be taken up by an inducible fructose EII PTS, which takes up also mannose ($EII^{Fru/Man}$) (Ajdić & Pham, 2007). Growth in sucrose can induce EII^{Fru} because the fructose moiety can be released from sucrose by the action of fructosyltransferases and further transported into the cell (Ajdić & Pham, 2007).

Lactose is transported and phosphorylated by the inducible EII^{Lac} PTS, generating intracellular lactose-phosphate which is further decomposed to galactose-6-phosphate and free glucose by the enzyme phospho-β-galactosidase (Calmes & Brown, 1979). Galactose-6-phosphate is further metabolized through the tagatose-6-phosphate pathway (Hamilton & Lebtag, 1979).

The second type of *S. mutans* sugar uptake system is, according to the genome sequence,

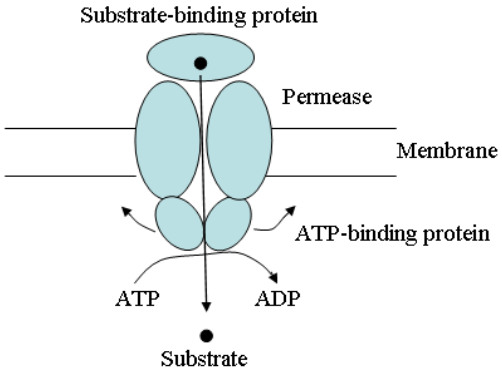

Figure 2. Schematic representation of the components of an ABC transporter (www.genome.jp).

represented by four ABC transporters (Ajdić *et al.*, 2002). An ABC transporter consists of 2 peripherally located ATP-binding proteins, 2 trans-membrane permeases and an extracellular substrate-binding protein anchored in the cell wall (**Figure 2**). *S. mutans* ABC transporters seem to be specialized in the transport of oligosaccharides (Ajdić & Pham, 2007). The *msm* operon encodes a system for transport and metabolism of multiple sugars, e.g. raffinose, melibiose and isomaltosaccharides. It consists of eight genes, which code for a sugar binding protein (MsmE), two permeases (MsmF and MsmG), an ATP-binding protein (MsmK), sucrose phosphorylase (GtfA), α-galactosidase and dextran glucosidase (DexB). In mutants where one of these *msm* components is mutated, the capacity of the bacterium to ferment isomaltose and isomaltotriose is abolished (Russell *et al.*, 1992). Maltosaccharide metabolism may be important for *S. mutans* because these molecules are substrates for the generation of intracellular polysaccharides used as storage compounds (Simpson &

Russell, 1998). The affinity of the *msm* system for a specific sugar being low (Russell *et al.*, 1992), the system has broad specificity for multiple sugars and therefore transport may only be significant when sugars are abundant. Like most microorganisms, oral streptococci are able to select a preferred sugar substrate when grown in a medium containing a mixture of sugars. However, the preferred sugar may differ from species to species. *S. mutans* prefers glucose over lactose when grown in a medium containing a mixture of these two sugars (Liberman & Bleiweis, 1984). A diauxic growth was also observed when *S. mutans* was grown in a mixture of glucose and sorbitol, where the former was the preferred sugar (Slee & Tanzer, 1983).

1.3.5 Initial sucrose-independent adhesion

The adherence of oral bacteria to the tooth surface is the first step for colonization. *S. mutans* initial adherence is mediated by a variety of surface-associated proteins such as the adhesin P1 (also called SpaP or antigen I/II), which binds to salivary proteins present in the pellicle on the tooth surface. Salivary agglutinin is a high-molecular-weight glycoprotein, which mediates the adhesion and aggregation of *S. mutans* via the cell wall-associated adhesin P1 (member of the AgI/II family of cell surface proteins) that is encoded by *spaP* (Jenkinson & Demuth, 1997). P1 interacts with fluid phase salivary agglutinin and mediates aggregation of *S. mutans* (Brady *et al.*, 1992; Loimaranta *et al.*, 2005), whereas adsorption of salivary agglutinin to solid surfaces provides a site for initial adhesion of the organism (Kishimoto *et al.*, 1989). Thus, salivary agglutinin is believed to facilitate bacterial discharge from the oral cavity or to promote colonization, depending on whether it is in solution or adsorbed on a surface (Jenkinson & Demuth, 1997).

1.3.6 Polysaccharide-mediated attachment

As mentioned above *S. mutans*' mediated damage to the teeth is associated almost exclusively with bacterial metabolism. Initially, virulence properties of the mutans streptococci were identified following comparison to other oral streptococci. One of the first virulence factors of the mutans streptococci identified by this approach was the ability to colonize smooth surfaces *in vitro* (Gibbons *et al.*, 1966), due to the capacity to produce extracellular polysaccharides from sucrose. *S. mutans* uses sucrose to synthesize α-1,3 and α-1,6 linked glucan polymers through the action of glucosyl transferases (Gtf), encoded by the *gtfB*, *gtfC* and *gtfD* genes. GtfB produces a water-insoluble glucose polymer called mutan, GtfD produces a water-soluble dextran, whereas GtfC produces a mixture of

water-soluble and water-insoluble glucans (Kuramitsu, 1993). The sticky nature of water-insoluble glucans facilitates the adherence of bacteria to the teeth and resists detachment by mechanical forces. *In vitro* studies have indicated that *gtfB* and *gtfC* are essential for the sucrose-dependent attachment of *S. mutans* cells to hard surfaces (Aoki *et al.*, 1986).

S. mutans produces also glucan-binding proteins, which have a role in sucrose-mediated attachment and contribute to its virulence. *S. mutans* has three glucan-binding proteins: GbpA, GbpB and GbpC. A *S. mutans* strain that lacks the *gbpA* gene showed both decreased sucrose-dependent adhesion and a lower total caries score in a rat model, whereas the deletion of *gbpC* lowered dextran-binding activity and sucrose dependent/independent adhesion (Matsumura *et al.*, 2003).

1.4. Microbial biofilm

1.4.1. General aspects

A biofilm is defined as a highly organized community of microorganisms embedded within a polymeric matrix, which is adherent to a living or inert surface (Costerton *et al.*, 1995). It is thought that single-cell organisms exhibit two distinct modes of behaviour. The first is the free floating or planktonic form, where the cells float or swim independently in a fluid (e.g. a liquid culture medium). The second is the biofilm mode, where cells are closely grouped and firmly attached to each other or the extracellular matrix forming a solid surface (Lappin-Scott & Bass, 2001). Changes in behaviour are triggered by factors such as quorum sensing and/or other mechanisms that vary between bacterial species. When cells switch from planktonic to a biofilm growth, they are thought to undergo a phenotypic shift in behaviour, resulting in the differential expression of large sets of genes (An & Parsek, 2007). Biofilms are characterized by surface attachment, genetic diversity, structural heterogeneity, complex interactions among microorganisms, and an extracellular matrix of polymeric substances. Biofilms grow on a range of surfaces including food, metallic objects, hard tissues and clinical instruments and they can act as reservoirs of infection. The biofilm growth mode offers protection against antimicrobial agents, such as antibiotics, thus biofilms are difficult to control and eradicate (Lappin-Scott & Bass, 2001).

1.4.2 Dental plaque as a biofilm

Dental plaque is a multispecies biofilm that accumulates in the oral cavity on surfaces such as the teeth, the tongue, the oral epithelia etc. (Rosan & Lamont, 2000). Oral biofilms are very

heterogeneous in structure and composition. Based on 16S rRNA nucleotide sequence analysis about 1000 different bacterial species or phylotypes have been identified in various forms of dental plaque. Approximately half of these cannot be cultured in the laboratory (Marsh, 2004; ten Cate, 2006).

Oral bacteria do not exist as independent entities in plaque; they function as co-ordinated, spatially organized and metabolically fully integrated microbial communities. A oral biofilm consists of bacterial microcolonies of various sizes and shapes with a pore-like extracellular matrix (Guggenheim *et al.,* 2001a; Guggenheim *et. al.,* 2001b; Thurnheer *et al.,* 2004). In oral supragingival biofilms, bacteria live under nutrient limitation most of the time and often in a dormant state (ten Cate, 2006).

The specific associations among various species of oral bacteria are believed to be driven by the production of surface adhesins that allow groups of species to co-colonize and by their ability to grow under the given biofilm conditions. These inter-bacterial associations may have evolved because of nutritional benefits or other advantages experienced by species growing in close vicinity to each other. An example of such cooperative behaviour has been seen between *S. mutans*, which produces large amounts of lactic acid, and *Veillonella parvula*, a consumer of lactic acid. Thus, there is likely a benefit to bacteria growing in dense biofilms and specific associations between certain species may be an essential factor in the persistence of these organisms in dental plaque (Lamont *et al.*, 2006).

The formation of dental plaque is a multistep mechanism. Distinct phases of development can be recognized (Marsh, 2004).

a) adsorption of host and bacterial molecules to the tooth surface;
b) passive transport of oral bacteria to the tooth surface;
c) co-adhesion of later colonizers to already attached early colonizers;
d) multiplication of attached microorganisms;
e) active detachment, enabling cells to colonize elsewhere.

After a tooth is cleaned, re-colonization by bacteria starts within minutes. The tooth is covered with a thin saliva pellicle, rich in proteins that provide an irreversible adhesion substrate for certain bacteria. The early colonizers of the supragingival biofilm are mitis group streptococci such as *Streptococcus oralis* and *Streptococcus sanguis*, which make up over 80% of the early biofilm. They provide attachment sites for the later colonizers, both Gram-positive and Gram-negative bacteria. The late colonizers attach to the biofilm bacteria and their extracellular matrix rather that to saliva-coated tooth surface. The succession of biofilm development involves coaggregation, coadherence and proliferation of oral bacteria, and if undisturbed, develops into a stratified, complex biofilm (Kreth *et*

al., 2009). After the initial colonization, the biofilm extends with the acquirement of additional Gram-negative rods, some of them being potential periodontal pathogens (Rosan & Lamont, 2000).

1.4.2.1 Quorum sensing and biofilm formation in *Streptococcus mutans*

Quorum sensing (QS) is a process by which bacteria communicate with each other. Using QS, bacteria not only sense the population density within their own species but also respond to neighbouring populations of other species (Podbielski & Kreikemeyer, 2004). QS allows the bacteria to modulate gene expression in response to the external conditions. In general, quorum sensing is regulated by signalling molecules that are produced in the cell and transported to the extracellular environment where they accumulate. The concentration of these molecules increases with growth of the bacterial population until it reaches a certain threshold. Bacteria are able to sense above threshold levels of the signalling molecules and respond with up-regulation of a series of regulatory pathways. Gram-negative bacteria use acyl-homoserine lactones (AHLs) as quorum sensing-signalling molecules, whereas Gram-positive bacteria use predominantly small peptides as inducers (Cvitkovitch, 2001).

S. mutans contains two different density-dependent signalling systems. The first is the intra-species quorum sensing system encoded by the *comCDE* genes, which regulates natural competence, biofilm formation, acid tolerance and bacteriocin production (Li *et al.*, 2001a; Li *et al.*, 2002b; van der Ploeg, 2005). The second is the interspecies signalling system mediated by the *luxS* gene (Merritt *et al.*, 2003).

***comCDE* system.** The discovery of a QS network in *S. mutans* was largely dependent on information derived from a closely related species, *Streptococcus pneumonie*, which comprises the best characterized cell-cell communication system within the genus *Streptococcus*. QS was first believed to serve mainly the acquisition and incorporation of foreign DNA from the environment. To this end it triggered the induction of a physiological state called genetic competence.

The *S. mutans com* quorum sensing pathway includes at least two loci – *comCDE* and *comAB*. The product of *comC* is a 46-amino acid peptide, whose C-terminus harbours the biologically active competence stimulating peptide (CSP), 21 amino acids in length. The *comAB* genes encode a secretion apparatus required for processing of ComC and export of CSP. ComA consists of an ATP-binding cassette transporter, which utilizes ComB as an accessory protein for cleavage of precursor CSP behind a Gly-Gly motif and export of CSP (Li *et al.*, 2001b).

The *comDE* genes encode a two-component signal transduction system comprising a

membrane-bound histidine kinase (ComD) and the response regulator (ComE). It is believed that when CSP reaches a certain threshold, it is detected by the ComD receptor, which undergoes phosphorylation at a conserved histidine residue. The phosphate is then transferred to the responder protein ComE, which becomes activated and then transduces the density-dependent message into a cellular response. It has been shown that addition of synthetic CSP to growing cultures of *S. mutans* increases the transformation efficiency, suggesting a role for the *comCDE* system in development of competence (Li *et al.*, 2001b). But unlike the situation in *S. pneumoniae*, there is no evidence that phosphorylated ComE directly activates *comX* gene, which encodes an alternative sigma factor essential for development of genetic competence. The mechanism by which the *comCDE* quorum sensing system of *S. mutans* regulates competence is at present unclear.

The *comCDE* genes are located upstream, but in the opposite direction of transcription, of the *bsmA* gene, which encodes a putative bacteriocin. The expression of *bsmA* (also called *nlmC*) was shown to be stimulated by CSP, but abolished in a *comDE* mutant, which suggested that the *comCDE* system is required for bacteriocin production. A number of other genes putatively encoding bacteriocins and associated immunity factors were also found to be regulated by this system (van der Ploeg, 2005).

LuxS system. While CSP-mediated signalling is involved in intra-species communication, autoinducer-2 (AI-2) molecules mediate inter-species communication. *S. mutans* produces AI-2 molecules in the phase of exponential growth (Merritt *et al.*, 2003). Autoinducer-2 production is mediated by the *luxS* gene, since the activity of AI-2 was abolished in *S. mutans* containing a mutation in the *luxS* gene. At the same time this mutation affected resistance to antibiotics, detergents and biofilm architecture when bacteria were grown in medium containing sucrose (Merritt *et al.*, 2003). This could be an indication that the *luxS*-mediated signal is involved in biofilm formation in *S. mutans* and may confer this bacterium an advantage over other resident bacteria of dental plaque.

1.4.2.2 Biofilms formed by *Streptococcus mutans*

When *S. mutans* grows in biofilms, both the competence and acid tolerance function are enhanced in comparison with planktonic cultures (Li *et al.*, 2001a). It was observed that genetic transformability was enhanced 10- to 100-fold in biofilm cells of *S. mutans* compared to their planktonic counterparts (Li *et al.*, 2001b). Biofilms formed of single mutants in *comC, comD, comE,* the sigma factor *comX*, or the triple mutant *comCDE* showed a different architecture in comparison to

the wild-type strain. Furthermore, the expression of *comCDE* was found to be up-regulated in biofilm-grown cells compared to liquid cultures (Li *et al.*, 2002b).

Besides QS-related genes, several other genes are associated with biofilm formation in *S. mutans*. Among them are as aforementioned the glucosytransferases (encoded by *gtfBCD)*, the fructosytransferase (*ftf*) and the glucan-binding proteins (*gbpBC*). In an attempt to study the influence of QS on the expression of these genes, it was shown that mutants in the two-component signal transduction system *comD* and *comE* have a positive regulatory effect on *gtfBCD*, *ftf* and *gbpB* (Senadheera *et al.*, 2007a). Also, in cultures of the wild-type strain supplemented with CSP the expression of *gtfB* and *gtfC* was up-regulated in a *comDE*-dependent manner compared to control cultures without CSP (Senadheera *et al.*, 2007b).

In addition to *comDE*, three more two-component signal transduction systems has been shown to be involved *in vitro* in *S. mutans* biofilm formation: *vicKRX*, *ciaRH* and hk11/rr11 (also known as *liaSR*). The *vicKR* system comprises a histidine kinase (VicK) and a response regulator, (VicR). VicK was shown to control the expression of *gtfBCD, ftf* and *gbpB*, whereas VicR seems to be essential for *S. mutans*. A *vicK* mutant was affected in biofilm architecture, cell growth and sucrose-mediated attachment (Senadheera *et al.*, 2005). Either deletion of *hk11* or *rr11* caused reduced biofilm formation, modified biofilm architecture, and resistance to acidic pH (Li *et al.*, 2002a). Inactivation of either *ciaR* or *ciaH* genes resulted in reduced biofilm biomass, whereas deletion of only the kinase *ciaH* altered sucrose-dependent biofilm formation (Qi *et al.*, 2004). Apart from two-component signal transduction systems, it was recently shown that the serine/threonine protein kinase PknB was also involved in biofilm formation. Upon detection of a signal, serine/threonine protein kinases are autophosphorylated at a conserved serine or threonine residue. The phosphate group can be transferred to target proteins, thereby modulating their activity. Mutations in *S. mutans pknB* gene resulted in altered biofilm structure, with the biofilm having a loose architecture resembling that of a biofilm in early stages of development (Hussain *et al.*, 2006).

1.5 Microarray technology

1.5.1 General aspects

A DNA microarray consists of a glass or plastic slide, which contains a collection of DNA probes arranged in a specific manner and attached onto the surface. The probes are designed to specifically bind during hybridization to their targets (Coppée, 2008). Each probe is unique and should

bind only to its target nucleic acid, which usually corresponds to a particular gene. To be able to quantify the signal, targets are labelled with a fluorescent dye. Upon hybridization, the intensity of the fluorescent signal reflects the degree of binding between target and probe.

There are several platforms for fabrication of nucleic acid hybridization-based microarrays. Data obtained from these platforms were found to be comparable (Barnes *et al.*, 2005; Woo *et al.*, 2004). In fact, with newly emerging, different array-based chips it is of great importance that these platforms should be comparable, allowing researchers to compare data across laboratories. Different platforms use different types of probes. They can be cDNA, short oligonucleotides (about 25-mer) or long oligonucleotides (longer than 50-mer). These probes can be deposited onto the arrays using ink-jet or contact-spot techniques, or they can be synthesized *in situ* on the slides. The method of detection differs between platforms; they can be based on dual-colour fluorescent dyes (called 2-colours arrays) or on antibodies (Chee *et al.*, 1996).

Because the microarray contains thousands of probes spotted on one slide, it is possible to quantify the transcriptome, which comprises the total number of RNA molecules that is expressed in the cell, tissue or organism, at one time (Blencowe *et al.*, 2009). Since their development in 1995 (Schena *et al.*, 1995), microarrays have been extensively used for such gene expression profiling to understand how different conditions affect transcription and how the transcriptome is regulated. A general work-flow of a microarray experiment for transcriptomics is depicted in **Figure 3.**

Typically, a microarray experiment generates a large amount of data. Therefore, it was mandatory that appropriate software programs emerged in order to extract the biological information. These programs were developed in parallel with the microarray platforms. Nowadays, there are multiple bioinformatic and statistical tools to deal with large data sets (Dresen *et al.*, 2003; Eisen *et al.*, 1998), but there is no such thing as a universal software which can be used between different platforms. However, for each software, features must exist for quality control, filtering and processing data as well as for extraction of biological meaning. All microarray data must comply with “Minimun information about microarray experiment“(MIAME) guidelines (Brazma, 2009).

1.5.2 Microarray as a tool for screening

In medical microbiology, microarrays have been used for a wide range of studies, for example for elucidation of mechanisms involved in host-pathogen interactions. Factors required for the bacteria to initiate infective colonization in their hosts, to persist and to adapt to this new environment have

been discovered using microarrays (My-Van La, 2008). But the transcriptome of host cells also undergoes changes upon infection (Hossain *et al.*, 2006). It was shown by microarray studies that *Salmonella thyphimurium* changes the transcriptional profile of infected macrophages and epithelial cells by inducing inflammatory responses (Eckmann *et al.*, 2000; Rosenberger *et al.*, 2000).
With microarrays it was also possible to identify changes in both gene and virulence factor expression in the pathogen, when the same host was exposed to different strains of the same pathogen (Xiang *et al.*, 1999). Moreover microarray analyses of changes in gene expression of a pathogen exposed to a new antimicrobial drug could not only assess the efficiency of the drug on a large spectrum of microorganisms (Freiberg *et al.*, 2005), but also determine the mechanisms by which the respective drug acts (Butcher *et al.*, 2006). Considering the rise of drug resistant pathogens in the last years, microarrays have been used to help understanding the mechanism of drug-resistance (Aakra *et al.*, 2005) or to perform drug resistance surveys (Collins *et al.*, 1989).

Apart from transcriptome studies, microarrays may also be used for diagnostic purposes. For example, microarrays have been developed to identify pathogenic bacteria from a mixed bacterial community (Kostic *et al.*, 2007). These "Diagnostic microarrays" are small-scale arrays containing specific, unique probes for each bacterium from the mixed community. Micorarrays have been developed also for the detection of human oral bacteria (Starke *et al.*, 2006) or for identification of bacteria in endodontic infections (Vianna *et al.*, 2005).

A similar approach was used to design "phylogenetic microarrays" for studying bacterial phylogeny and for identification of new strains (Huyghe *et al.*, 2008). The probes were designed using 16S rRNA sequences to match the phylogenetic nodes at every level in the phylogenetic tree.

With *S. mutans,* there have been several studies with DNA microarrays: screening for genes that are controlled by two-component signal transduction systems involved in the oxidative stress response during growth in biofilms (Perry *et al.*, 2008), identification of genes involved in biofilm formation (Wen *et al.*, 2006), sugar uptake (Ajdić & Pham, 2007) and acid tolerance (Lemos *et al.*, 2008). Comparative transcriptomics has also been employed for detection of *S. mutans* genes whose expression is induced by CSP (Perry *et al.*, 2009) or autoinducer II (Sztajer *et al.*, 2008) or after transition from an anaerobic environment to an aerobic environment (Ahn *et al.*, 2007). Finally, transcriptome analysis has been carried out to identify genes which are differentially expressed in biofilms compared with planktonic cultures (Shemesh *et al.*, 2007b).

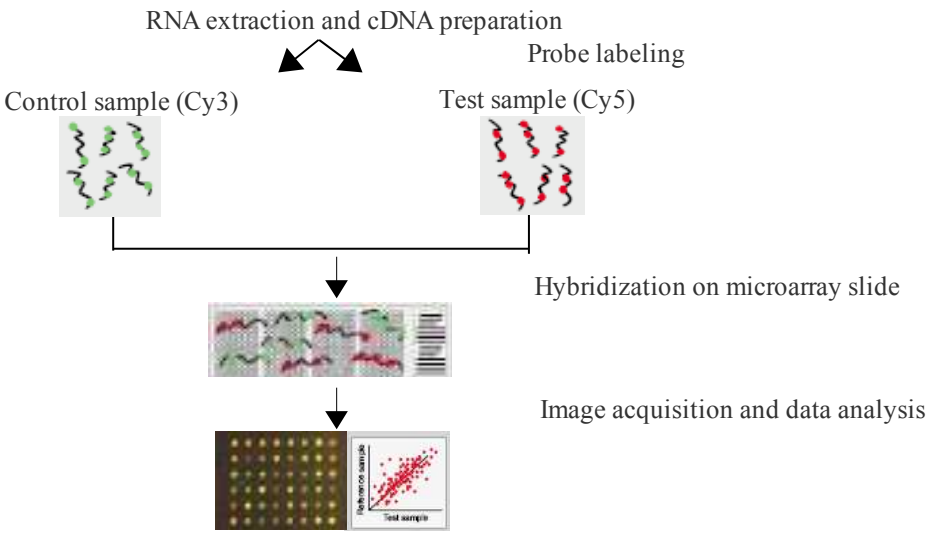

Figure 3. Scheme of transcriptome analysis using DNA microarray technology: probe preparation and hybridization, image acquisition and data analysis (modified from Cook & Sayler, 2003).

1.6 Outline of the work

Although *S. mutans* is an "obligate" biofilm bacterium, up till now most studies concerning the organism have been carried out with planktonically grown bacteria. But it is generally understood that microorganisms that grow in biofilms show a different pattern of gene expression than those that are grown in planktonic cultures. Part I of the results describes how gene expression is modulated when *S. mutans* grows under different biofilm conditions. Transcriptome analyisis was used to examine the transcriptomic differences between planktonic growth and biofilm growth. Moreover, since *S. mutans* is subject to changes in nutrient availability, the influence of nutrient starvation was investigated.

Part II of the results concerns with the characterization of two gene clusters, *lrgAB* and *cidAB*, and a two-component signal transduction system (*lytRS*) located upstream of *lrgA*. The *lrgAB* genes were found to be differentially expressed in four out of six microarray experiments described in Part I of the results. Analogous to the situation in *S. aureus*, these genes might be involved in cell death and lysis. To investigate their possible function, the expression of *lrgA, cidA* and *lytR* was studied and the phenotype of mutants in *lrgAB*, *cidAB* and *lytSR* and *lytR* were examined.

Part III of the results describes the phenotypical analysis of *S. mutans* strains lacking the eukaryotic serine-threonine kinase (encoded by the *pknB* gene) and/or its cognate phosphatase (encoded by the *pppL* gene). It was previously shown that a *pknB* mutant displays a decreased growth rate, formed biofilms of different architecture than the wild type and sensitivity to acidic pH (Hussain *et al*., 2006). In the present study, further phenotypic investigations of *pknB*, *pppL* and *pknB pppL* double mutants were carried out. Microarray analysis was used to analyze the transcriptome of the *pknB* mutant. Further investigations of the mutants included assays on bacteriocin production, transmission electron microscopy for morphological characterization of the cells, the capacity of *pknB* mutant to withstand oxidative stress, and the expression of bacteriocins in two-component signal transduction systems which may interact with PknB.

II. MATERIALS AND METHODS

2.1 Materials

All chemicals used were purchased from Sigma-Aldrich or Fluka (Buchs, Switzerland). Oligonucleotides were synthesized by Microsynth (Balgach, Switzerland) and Sigma. Restriction enzymes were purchased from Fermentas (Vilnius, Lithuania) and New England Biolabs (Beverly, MA, USA).

2.2 Bacterial strains, media and growth conditions

Bacterial strains and plasmids are listed in **Table 1**. *S. mutans* strains were routinely grown at 37°C in THYE medium, which consists of Todd-Hewitt (Becton-Dickinson, Le Pont du Claix, France) with 0.3% yeast extract (Oxoid, Basingstoke, Hampshire, England) or a biofilm medium (BM) modified from (Loo *et al.*, 2003) containing 0.8% glucose or sucrose as the carbon source. BM consisted of 53 mM K_2HPO_4, 15 mM KH_2PO_4, 10 mM $(NH_4)_2SO_4$, 35 mM NaCl, 2 mM $MgSO_4$, 0.2% (wt/vol) Casamino acids,, and was supplemented with filter sterilized vitamins (0.04 mM nicotinic acid, 0.1 mM pyridoxine HCl, 0.01 mM pantothenic acid, 1 µM riboflavin, 0.3 µM thiamine HCl, and 0.05 µM D-biotin) and amino acids (1 mM L-arginine HCl, 1.3 mM L-cysteine HCl, 4 mM L-glutamic acid, and 0.1 mM L-tryptophan). Vials were incubated under aerobic or anaerobic conditions (85% N_2, 10% CO_2 and 5% H_2) or in an atmosphere of 10% CO_2 and 90% air. When required, antibiotics were added at the following concentrations: erythromycin (15 µg/ml) and kanamycin (750 µg/ml). *Escherichia coli* strain JM109 was used as host for the propagation of plasmids and grown in LB medium at 37°C with aeration. When required, antibiotics were added at the following concentrations: erythromycin (400 µg/ml) and kanamycin (50 µg/ml).

2.3 Isolation of nucleic acids

Chromosomal DNA from *S. mutans* was isolated using the GenElute bacterial genomic DNA kit (Sigma, Buchs, Switzerland). Plasmid DNA was isolated using the Jetstar 2.0 Plasmid midiprep kit (Genomed GmbH, Löhne, Germany).

For RNA extraction, cells were centrifuged for 15 min at 2800 x g and 4°C. Total RNA was extracted form cell pellets using an RNeasy Mini Kit (Qiagen, Hilden, Switzerland) with an on-column

RNase-free DNase digestion step according to the manufacturer's recommendation. All RNA samples were checked for integrity by denaturing formaldehyde agarose gel electrophoresis and by electrophoresis on a 2100 Bioanalyzer (Agilent, Santa Clara CA, USA).

2.4 Polymerase chain reaction (PCR)

PCR was used to amplify DNA fragments for cloning purposes or for verifying mutant strains. A standard PCR reaction mixture of 100 µl contained 100 pmoles of both primers, 0.2 mM dNTPs, a suitable amount of template DNA (about 100 ng) and 0.2 U of Taq polymerase in the buffer supplied by the manufacturer (New England Biolabs, Ipswich, USA). For amplification of fragments for cloning, Vent DNA polymerase (2 U, New England Biolabs) was employed. Standard PCR conditions consisted of an initial denaturation for 3 min at 95°C, followed by 25 or 30 cycles composed of denaturation at 95°C for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 1 min. When required, annealing temperature, number of cycles and elongation time were adjusted.

2.5 Transformation of *S. mutans*

For transformation, *S. mutans* was grown overnight in THYE supplemented with 5% heat-inactivated human serum. The next day, a part of the culture was diluted 1:40 in 5 ml of fresh, pre-warmed THYE /human serum and incubated standing undisturbed at 37°C for 3.5 hours. Thereafter, 0.5 ml of this culture was mixed with 0.5 µg of linearized plasmid and further incubated for one hour. Subsequently, 0.3 ml from the mixture was plated onto THYE agar containing the appropriate antibiotic for selection. Plates were incubated anaerobically for two days.

2.6 Construction of *lrgAB*$^-$, *cidAB*$^-$, *lytR*$^-$ and *lytSR*$^-$ mutants

For generation of an *lrgAB* mutant, a 670-bp fragment containing the 3`end and a segment downstream of the *lrgB* gene was amplified by PCR with primers lrgAB3 (5'-GAT CCT CGA GGT GTT TCA TAA TAC GAA CGA-3'; restriction site underlined) and lrgAB4 (5'-GAT CGA ATT CAT AGA AGC AGC AAC ACA-3'), digested with restriction enzymes NcoI and EcoRI and cloned downstream of the erythromycin resistance gene of the plasmid pFW15 to give plasmid pOMZ380. A second 879-bp fragment containing the 5`end and the region upstream of *lrgA* was amplified by PCR using primers lrgAB1 (5'-GAT CGG ATC CTA ATT CAG CAG AGT AAG G-3`) and lrgAB5 (5'-CGT

AGA GCT CGT GTT TCA TAA TAC GAA CG-3'), digested with restriction enzymes BamHI and SacI and cloned upstream of the erythromycin resistance gene in the plasmid pOMZ380 to give pOMZ381. pOMZ381 was linearized with BamHI and subsequently transformed into *S. mutans* OMZ1001. Since plasmid pFW15 and its derivatives cannot replicate in *S. mutans*, erythromycin resistant colonies which arise should have integrated the insert after a double crossing-over event. One colony was selected and named OMZ 1083.

For generation of a *cidAB* mutant, a 528-bp fragment containing the 3`end and the region downstream of the *cidB* gene was amplified by PCR with primers cidAB1 (5'-GAT CGA GCT CTA AGA TTA AGT GGC TGA TTC-3') and cidAB2 (5'-GAG CAA GCT TAA TAA TCA TCA ACT GCA CAT A-3'), digested with restriction enzymes SacI and HindIII and cloned downstream of the erythromycin resistance gene of the plasmid pFW15 to give plasmid pOMZ390. A second 583-bp fragment containing the 5`end and a segment upstream of *cidA* was amplified by PCR using primers cidAB3 (5'-GCG CCT GCA GTT AAA ATG ACT TTT AAT CAA CCA-3') and cidAB4 (5'-GAT CGA ATT CTG TCT GTA AGC CTA ACT C-3'), digested with restriction enzymes PstI and EcoRI and cloned upstream of the erythromycin resistance gene in the plasmid pOMZ390 to give pOMZ391. pOMZ391 was cut with BamHI and introduced in *S. mutans* OMZ1001 by transformation. One erythromycin resistant colony was selected and named OMZ1084.

For generation of a *lytR* mutant, a 604-bp fragment containing the 3`end and a segment downstream of the *lrgA* gene was amplified by PCR with primers lytRS3 (5'-GCT GCC ATG GAC CTT ATA AAT GAA CTA GTT TC-3') and lytRS4 (5'-GAT CGA ATT CTT GCC ACA TTC ACC TTT-3'), digested with restriction enzymes NcoI and EcoRI and cloned downstream of a erythromycin resisting gene of the plasmid pFW15 to give plasmid pOMZ443. A second 711-bp fragment containing the 5` end and a segment upstream of *lytR* was amplified by PCR using primers lytRSBam (5'-GAT CGG ATC CTA TTG CCG AGG AAC-3`) and lytRSSac (5`-GAT CGA GCT CTC TTG TCT TGC CAA CAT-3`), digested with restriction enzymes BamHI and SacI and cloned upstream of the erythromycin resistance gene in the plasmid pOMZ443 to give pOMZ444. pOMZ444 was digested with BamHI and introduced into *S. mutans* OMZ1001 by transformation and selection for erythromycin resistance. One colony was selected and named OMZ1108. All mutants were verified by PCR analysis and sequencing.

For construction of a *lytSR* double mutant, a 558-bp fragment including the 5` end and a fragment upstream of *lytS* gene was amplified by PCR with primers lytRS1 (5`- GAT CGG ATC CCA

GAT AAA CTC GTT CCA AAT-3' and lytRS2 (5`-GGT CGA GCT CCA TTA ACA TAT TAT GCT TCT CC-3'), digested with BamHI and SacI and cloned in plasmid pFW15 to give plasmid pOMZ395. Plasmids pOMZ395 and pOMZ444 were both digested with BamHI and SacI, purified and ligated together to give pOMZ445. pOMZ445 was linearized with BamHI and transformed into *S. mutans* OMZ1001. Colonies were selected on THYE plates containing erythromycin. One colony was selected and named OMZ1109.

2.7 Preparation of samples for microarray experiments

S. mutans strain OMZ 918 (UA159) was used for all microarray experiments. Unless stated otherwise, biofilms were rinsed twice with cold 0.9% NaCl, scraped from the surface of the bottle and resuspended in 1:2 THYE/RNAprotect Bacteria Reagent (Qiagen, Hilden, Germany) for RNA stabilization. After centrifugation (15 min, 3700 rpm and 4°C), the supernatant was discarded and the pellet was kept at -20°C until RNA extraction.

Exponential and stationary phase *in vitro* biofilms and planktonic culture (microarrays 1 and 2)

For generation of exponential biofilms (microarray 1), bacteria were grown anaerobically at 37°C. Overnight planktonic cultures of *S. mutans* were diluted 25-fold in THYE and allowed to grow planktonically until OD_{600}=1.5. Cells were then diluted 100-fold in THYE medium and cultured for 4 hours in 175 cm^2 *EasyFlask* cell culture bottles (Nunc, Wiesbaden, Germany). Planktonic cultures were prepared by 100-fold dilution of an overnight culture (OD_{600}=1.4) in THYE until the OD_{600} reached 0.3 (exponential phase).

For comparison of stationary phase cultures (microarry 2), both biofilm and planktonic cultures were prepared as above, but biofilms were harvested after 16 hours of growth (stationary biofilm) and planktonic cultures were grown until OD_{600}= 2 (approximatively 7 hours).

Biofilms in medium supplemented with glucose or sucrose (microarrays 3 and 4)

Biofilms were generated as for exponential biofilm cultures, but THYE medium was supplemented with either 0.3% glucose or 0.5% sucrose. The biofilms were grown for 4 hours (exponential growth phase, microarray 3) or 16 hours (stationary growth phase, microarray 4).

Starved and fed biofilms (microarrays 5 and 6)

Biofilms were grown anaerobically at 37°C in 24-well polystyrene cell culture plates on bovine enamel disks (aprox. 6 mm in diameter). Before the beginning of the experiment, disks were incubated with whole, unstimulated saliva (0.8 ml) for 4 hours to generate a pellicle (Guggenheim *et al.*, 2001a). Disks were covered with 1.6 ml of growth medium consisting of 1 ml FUM medium (Gmür & Guggenheim, 1983) with 0.3% glucose, 0.4 ml saliva and 0.2 ml of *S. mutans* cell suspension (OD_{600}=1) in FUM medium and incubated for 45 minutes to promote adhesion of the bacteria. The discs were washed three times in 2 ml 0.9% NaCl to keep only attached cells and further incubated in saliva only. After 16.5, 20.5, 24.5, 40.5, 44.5 and 48.5 hours, discs were incubated for 45 minutes in 1.6 ml of a mixture of 1 ml FUM with 0.15% glucose and 0.15% sucrose and 0.6 ml saliva and then returned to saliva. Saliva was replaced at 16.5 and 48.5 hours. These alternations of feeding and starving periods mimics the "fast and feast" periods of *S. mutans* in its natural environment (van der Ploeg & Guggenheim, 2004).

For comparison of starved biofilms with fed biofilms (microarray 5), half of the biofilms that had been in saliva for about 16 hours after the last feeding (designated as "starved") were harvested after 64.5 h. The other half was subjected to one more feeding for 45 minutes and than immediately harvested ("fed biofilms"). To obtain re-starved biofilms, a portion of fed biofilms was returned back into saliva and harvested after 45 minutes. Re-starved biofilms were compared with fed biofilms in microarray 6.

For harvesting, biofilms were washed 3 times in 0.9% NaCl, vortexed for 2 minutes, sonicated for 10 seconds, resuspended in RNAprotect Bacteria Reagent and kept at -20°C until RNA extraction.

Comparison of gene expression between wild type strain and *pknB* mutant (microarray 7)

Overnight cultures of the *S. mutans* wild-type strain (OMZ918) and the *pknB* mutant (strain PKNB) were diluted 100-fold in THYE and grown until OD_{600}=0.3 (exponential growth phase). 5 ml of each culture was mixed with 1:2 (v/v) THYE/RNAprotect Bacteria Reagent (Qiagen), centrifuged and the pellet was kept at -20°C until RNA extraction.

2.8 Characteristics of microarrays

S. mutans microarray slides were obtained from Dr. Georg Conrads (RWTH Aachen University,

Aachen, Germany). For each *S. mutans* gene, one 50-mer oligonucleotide probe had been designed. The oligonucleotides (MWG Biotech, Ebersberg, Germany) were spotted in triplicate on CodeLink activated slides (Amersham Biosciences) at a concentration of 25 µM in 1.5 × sodium phosphate buffer. As negative control for hybridization, five *Arabidopsis thaliana* sequences were included.

2.9 cDNA preparation, hybridization conditions and washing, scanning and data mining.

For each experiment, at least three biological replicates were used. One of these replicates was carried out with a dye swap to account for dye biases. 7 µg of total RNA was reverse transcribed and cDNA was labelled using CyScribe Post-Labelling (GE Healthcare). This process was done in two steps. First, aminoallyl-dUTP nucleotides were incorporated into cDNA during the reverse-transcription reaction, and second, the aminoallyl-dUTP-containing cDNA was labelled with fluorescent dyes Cy3 (green) and Cy5 (red). For the reverse-transcription reaction, 7 µg of total RNA was mixed with 3 µl random nanomers and incubated at 70°C for 5 minutes. The reactions were cooled down at room temperature for 10 minutes and further on ice and mixed with 4 µl 5x CyScript buffer, 2 µl DTT, 1 µl Nucleotide mix, 1 µl aminoallyl-dUTP and 1 µl CyScript reverse transcriptase. The samples were incubated at 42°C for 1.5 hours. For the degradation of mRNA from the single-stranded cDNA, 2 µl of 2.5 M NaOH were added to each cDNA reaction and incubated at 37°C for 15 minutes. To neutralize the reaction, 10 µl of 2 M HEPES (free acid) solution were added and the cDNA was purified using an Illustra™ CyScribe GFX Purification kit (GE Healthcare). For the labelling procedure, the purified aminoallyl cDNA was mixed with ready-to-use aliquoted Cy3 or Cy5 dyes and incubated at room temperature in the dark for 1.5 hours. To inactivate any unreacted CyDye, 15 µl of a 4 M solution of hydroxylamine were added to each coupling reaction and incubated in the dark for 15 minutes. The labelled cDNA was further purified like the cDNA obtained from the reverse transcription reaction. Approximately 500 ng of each Cy3- and Cy5-labeled cDNA were used for hybridization on each chip. Hybridization and washing were performed in a fully automated HS 4800™ Pro hybridization station (Tecan, Männedorf, Switzerland). Washing buffers were as follows: buffer 1: 2 x SSC, 0.5% SDS; buffer 2: 0.5 x SSC, 0.5% SDS and buffer 3: 0.1 x SSC. 20X SSC stock solution (3 M NaCl and 0.3 M Sodium Citrate) was used for preparing washing buffers.

The arrays were scanned using an Agilent DNA microarray scanner (Agilent Technologies, Basel, Switzerland) at full laser power. GeneSpotter software (MicroDiscovery GmbH, Berlin, Germany) was used for determining the average signal intensity and the local background for each

spot. The data were then imported into the program Genespring (Agilent Technologies, Basel, Switzerland), normalized using the Lowess normalization method and filtered leaving out intensities below 100 in both channels. The statistical significance of ratios was determined by applying the Student t-test. Genes were considered to be differentially expressed if the ratio was above 2 with a p-value ≤ 0.05.

2.10 RNA quantification with quantitative RT-PCR

Quantitative RT-PCR was performed with the same RNA samples that were used for microarray experiments. The gene-specific primers were designed using PrimerExpress software (Applied Biosystems, Rotkreuz, Switzerland). RNA samples were treated with DNase (Promega, Wallisellen, Switzerland) before reverse-transcription. A reverse-transcription reaction mixture containing 500 ng total RNA, 200 ng random hexamers and 10 mM dNTPs was incubated at 65°C for 5 minutes and chilled on ice for at least 1 minute. Then, 4 µl of 5x First-Strand Buffer, 1 µl of 0.1 M DTT, 1 µl of RNaseOUT and 200 Units of SuperScript III RT (Invitrogen, Basel, Switzerland) were added to each reaction mix. The reactions were incubated at 25ºC for 5 minutes and then at 50ºC for one hour. Reactions were inactivated by incubation at 70ºC for 15 minutes. DNA-control reactions, without reverse transcriptase enzyme, were run in parallel to check for DNA contamination. The real-time PCR reaction mixture (25 µl) contained SYBR Green PCR master mix (Applied Biosystems), 2.5 ng cDNA, and 50 nM of the specific forward and reverse PCR primers (**Table 5, Appendix**). PCR conditions consisted of an initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of denaturation at 95ºC for 15 s, and annealing and extension at 60ºC for 1 minute. A dissociation curve was generated for each primer pair to exclude that primer-dimer formation had occurred. Reactions were run on an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The expression level of the selected genes was normalized using the 16S rRNA transcript of *S. mutans* as an endogenous control. Each assay was performed with at least 2 independent RNAs in duplicate.

2.11 Bacteriocin assay

Bacteriocin production by the *S. mutans* wild-type strain and by the mutants PKNB, PPPL, and PKPL were performed as described before (van der Ploeg, 2005). Briefly, overnight cultures of the above mentioned strains were stab-inoculated in THYE agar and grown in a 10% CO_2 atmosphere at 37°C for 24 hours. 100 µl of an overnight culture of the indicator strain *Enterococcus faecalis* OMZ940

was mixed with 4 ml molten THEY soft agar and spread over the THYE plates. The plates were incubated in 10% CO_2 for 24 hours and the diameters of zones of inhibition were measured for each strain.

2.12 Influence of paraquat on growth

Overnight cultures of the wild strain and the mutant PKNB and wild-type were diluted 25-fold in fresh THYE to give an OD_{600} of 0.05. The cells where then exposed to 25 mM of paraquat and aliquated in 96-well microtiter plates. The optical density at 600 nm was measured every hour over a period of 22 hours at 37°C in a Spectramax M2 instrument (Molecular Devices, Sunnyvale, California). Controls without paraquat as well as water controls were run in parallel.

2.13 Electron microscopy

Strains UA159, PKNB, PPPL and PKPL were grown in THYE broth to the mid-exponential phase (OD_{600}=0.3). Growth was arrested by fixation in 2.5% glutaraldehyde for one hour, whased in 0.2% cacodylate buffer, centrifuged, followed by post fixation in 1% osmium tetraoxide / 0.1 M cacodylate buffer for one hour. Dehydration was done using a graded series of ethanol. Propylene oxide was then used to facilitate the infiltration with Epon. Ultrathin sectioning was done with a Reichert-Jung Ultracut E ultramicrotome; the sections were mounted on uncoated 400 mesh copper grids. Samples were first contrasted with saturated, acidified uranyl acetate as described by Tandler, (1990), omitting the addition of methanol, and then with lead citrate as described by Venable & Coggeshall (1965), both for 3 minutes each. The sections were viewed on a Philips EM400T TEM at 60 kV.

2.14 Long-term survival of *S. mutans*

Overnight cultures of *S. mutans* strains were brought to the same optical density (about 1.5 at OD_{600}), diluted 20-fold in THYE and incubated aerobically at 37°C. Samples were removed at different time points (1, 2, 3, 4, 6, 9, 11 and 13 days), sonicated for 10 seconds and dilutions were plated onto THYE agar plates. After 24 hours of incubation at 37°C, the number of colonies was counted.

2.15 Exposure of *S. mutans* to weak acids

An overnight culture of the strain OMZ1001 was diluted to a OD_{600}=0.1 and grown anaerobically for 3 hours before the addition of 30 mM of either lactic acid, acetic acid, pyruvic acid or formic acid. As control, the pH of one culture was adjusted to pH 5.0. The cultures were exposed to the acids for one hour. Subsequently, 2 ml of cells were mixed with 4 ml of RNAprotect Bacteria Reagent/THYE and pelleted. The pellet was kept at -20°C until RNA extraction (protocol adapted from (Patton *et al.*, 2006).

2.16 Exposure of *S. mutans* to carbonyl cyanide m-chlorophenyl hydrazone (CCCP).

Overnight cultures of *S. mutans* were diluted to an OD_{600} of 0.1, grown at 37°C until an OD_{600} of about 0.8 and split into two equal fractions (3 ml each). To one of each of the fractions, 3 µl of 100 mM CCCP in dimethylsulfoxide was added to give a final concentration of 100 µM. As control, the other fraction was supplemented with the same amount (3 µl) of dimethylsulfoxide. All samples were then further incubated anaerobically for 30 minutes, mixed with 2:1 (v/v) RNAprotect Bacteria Reagent/THYE and centrifuged. The pellet was kept at -20°C until RNA extraction (protocol adapted from (Patton *et al.*, 2006).

2.17 Quantification of chain length

Overnight cultures of *S. mutans* strains were 10-fold diluted in THYE and 5 µl of each culture was deposited onto a microscopic glass slide and allowed to dry. The bacteria were fixed with 2.5% glutaraldehyde for 5 minutes, washed in TE buffer (10 mM Tris, 1 mM EDTA, pH=8.0) and dried again. Slides were inspected optically with a phase-contrast microscope (Olympus BX61) at a magnification of 1000. Images were acquired from the microscope with an F-View II camera (Olympus) and visualized using the Cell^{p} software version 2.3 (Built 1121) from Olympus. Over 200 bacterial chains from at least 3 biological replicates were counted on prints of digital images.

2.18 Acid sensitivity assay

Overnight cultures of strains were brought to an OD_{600} of 1.5. 20 µl of serial dilutions of each culture (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) were dropped onto plates containing THYE agar at pH=7.0 or pH=5.0. The plates were incubated in a CO_2 atmosphere for 24 hours at 37°C. The plates were inspected for the size of the colonies as well as for a reduction on the colony numbers.

2.19 Quantification of *S. mutans* from biofilms

Biofilms were prepared as those for microarray experiments, except that they were grown in 96-well plates (TPP, Trasadingen, Switzerland). After incubation, the supernatant was discarded. Biofilms were washed twice with ice-cold distilled water and dried for 2 hours at room temperature. Subsequently, 50 µl of 0.1% crystal violet were added in each well. The plates were incubated at room temperature with gentle shaking for 15 minutes. The biofilms were washed to remove excess staining solution and dried. Biofilm-bound crystal violet was extracted with 30% acetic acid and the OD_{600} of the resulting solution was measured. Data are provided as average of triplicates.

Table 1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Features	Source or reference
Strains		
Escherichia coli		
JM109	used for the propagation of plasmids	New England Biolabs
Streptococcus mutans		
OMZ918	wild-type UA159, ATCC 700610	ATCC
OMZ1001	derivate of UA159, high transformation frequency	Laboratory collection
OMZ1083	as OMZ1001 but *lrgAB* ::*ermAM*	This study
OMZ1084	as OMZ1001 but *cidAB* ::*ermAM*	This study
OMZ1108	as OMZ1001 but *lytR* ::*ermAM*	This study
OMZ1109	as OMZ1001 but *lytSR* ::*ermAM*	This study
PKNB	UA159 Δ*pknB* ::*aphA3*, Km^r	Hussain *et al.,* 2006
PPPL	UA159 Δ*pppL* ::*erm*, Erm^r	E. Allan, UCL Eastman Dental Institute
PKPL	UA159 Δ*pknB* ::*aphA3*, Δ*pppL* ::*erm*, Km^r, Em^r	E. Allan, UCL Eastman Dental Institute
OMZ955	UA159 *hk11*::*ermAM*	D. Cvitkovitch, University of Toronto
OMZ953	UA159 *vicK* ::*ermAM*	D. Cvitkovitch, University of Toronto
OMZ1110	UA159 *ciaRH* ::*erm*	This study
OMZ1111	UA159 *comDE* ::*erm*	This study
OMZ1112	UA159 *pknB* ::*aphA3 ciaRH* ::*erm*	This study
Enterococcus faecalis		
OMZ940	CG110	Gawron-Burke & Clewell, 1984
Plasmids		
pFW15	replacement vector, Erm^r	Podbielski *et al.,* 1996
pFW13	replacement vector, Erm^r	Podbielski *et al.,* 1996
pOMZ42	used for construction of *ciaRH* ::*ermAM* mutant	This study
pOMZ61	used for construction of *comDE* ::*ermAM* mutant	This study
pOMZ381	used for construction of *lrgAB* ::*ermAM* mutant	This study
pOMZ391	used for construction of *cidAB* ::*ermAM* mutant	This study
pOMZ444	used for construction of *lytR* ::*ermAM* mutant	This study
pOMZ445	used for construction of *lytSR* ::*ermAM* mutant	This study

III. RESULTS AND DISCUSSION

Part I. Gene expression in *Streptococcus mutans* biofilms

To explore the physiology of *S. mutans* in biofilms, the biofilms were grown under different environmental conditions. Gene expression profiles of *S. mutans* grown in *in vitro* biofilms were compared with those of planktonic cultures, both in the exponential and stationary phase of growth. Changes in the transcriptome induced by the presence of either glucose or sucrose as a carbon source in exponentially growing and mature biofilms were analysed. In addition, the response of *S. mutans* to starvation and feeding was compared.

3.1.1 Gene expression in exponential phase biofilms (Microarray 1)

The aim of this experiment was to determine whether the transcriptome of *S. mutans* in biofilms is different from that in planktonic cultures during the exponential growth phase. The biofilm cultures were grown for 4 hours in cell culture flasks, whereas the planktonic culture was grown to an OD_{600} of 0.3.

When comparing the transcriptome of *S. mutans* grown in biofilms with that of the planktonic culture in exponential phase, 12 genes were found to be up-regulated and 26 down-regulated in biofilms (**Table 2**). The validity of the microarray data was examined using quantitative RT-PCR (qRT-PCR). From the 11 selected genes, the differential expression of 8 genes could be confirmed (**Figure 4**). A fair correlation (R^2=0.56) was observed between the ratios obtained from microarray and those obtained from qRT-PCR.

Among the small number of up-regulated genes in biofilms was a set of three genes which encodes an ABC-type Mn^{2+} and Fe^{3+} transport system. The operon comprises three genes: SMU.182 (*sloA*), an ATP-binding protein, SMU.183 (*sloB*), a membrane component of a putative ABC transporter and SMU.184 (*sloC*), a substrate-binding protein. The product of the *sloC* gene is thought to have a dual function: as an adhesin as part of the LraI family of lipoproteins and as a cell surface ligand for metallic ions (Fenno *et al.*, 1995; Paik *et al.*, 2003). This suggests that the requirement for manganese or other metal ions is higher in exponentially growing biofilms than in exponentially growing planktonic cultures. This is supported by the observation that the expression of SMU.770c, annotated as putative manganese transporter, was also up-regulated 2.1-fold in biofilms. Moreover,

expression of genes encoding proteins which required divalent cations for activity (*sod*, SMU.1784c) was down-regulated in biofilms. A *sloA* mutant showed loss of virulence in a rat model of endocarditis, suggesting that metal transport is required for *S. mutans* to establish biofilms on heart valves (Paik *et al.*, 2003).

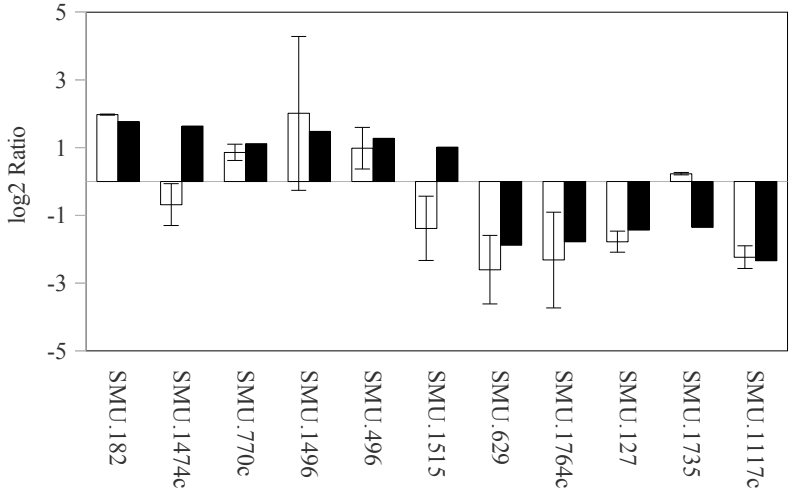

Figure 4. Validation of microarray 1 results with qRT-PCR. Data were generated from two independent RNA preparations, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratios.

The most down-regulated gene in biofilms was SMU.1117, encoding H_2O forming NADH oxidase. This enzyme catalyses the 4-electron reduction of oxygen to form water and may be important for the bacterium to reduce the oxygen level in order to prevent the formation of reactive oxygen species (Higuchi *et al.*, 1999). Although Nguyen et *al*. (2002) showed that under aerobic conditions NADH oxidase activity is lower in biofilms compared with planktonic cultures of *S. mutans*, it is difficult to explain why this would also be the case under anaerobic conditions. Among the down-regulated genes, the gene encoding a cell surface antigen, *spaP*, showed a 2.29-fold decrease in expression. The SpaP protein is often credited to promote sucrose-independent adhesion (Arirachakaran *et al.*, 2007). It was observed that in biofilm cultures the expression of *spaP* showed a slight decrease in the presence of manganese. It can be speculated that down-regulation of sucrose-independent adhesins within the biofilm could indicate they are not needed for biofilm maintenance (Arirachakaran *et al.*, 2007). The *gtfB* gene, encoding a glucosyltransferase required for the synthesis of extracellular water-insoluble glucans (Kenney & Cole, 1983), was down-regulated 2.38-fold.

Several genes from a gene cluster involved in response against attacks of bacteriophage or

plasmid (SMU.1764c – SMU.1754c) (van der Ploeg, 2009), were down-regulated in biofilm cultures. Several genes located within transposon TnSmu1 (SMU.198c, SMU.201c, SMU.202c, SMU.205c and SMU.206c) were also down-regulated in biofilm cultures.

3.1.2 Gene expression in stationary phase biofilms (Microarray 2)

Microarray data analysis revealed that, compared to biofilms composed of exponentially growing cells, many more genes were differentially expressed in stationary phase biofilms. A total of 17.7% of all genes were found to be differentially expressed (180 up-regulated and 167 down-regulated). A full list of all genes which are differentially expressed in stationary phase biofilms compared with stationary phase planktonic culture is provided in **Table 1** of the **Appendix**. The genes were grouped in functional categories (**Figure 5).**

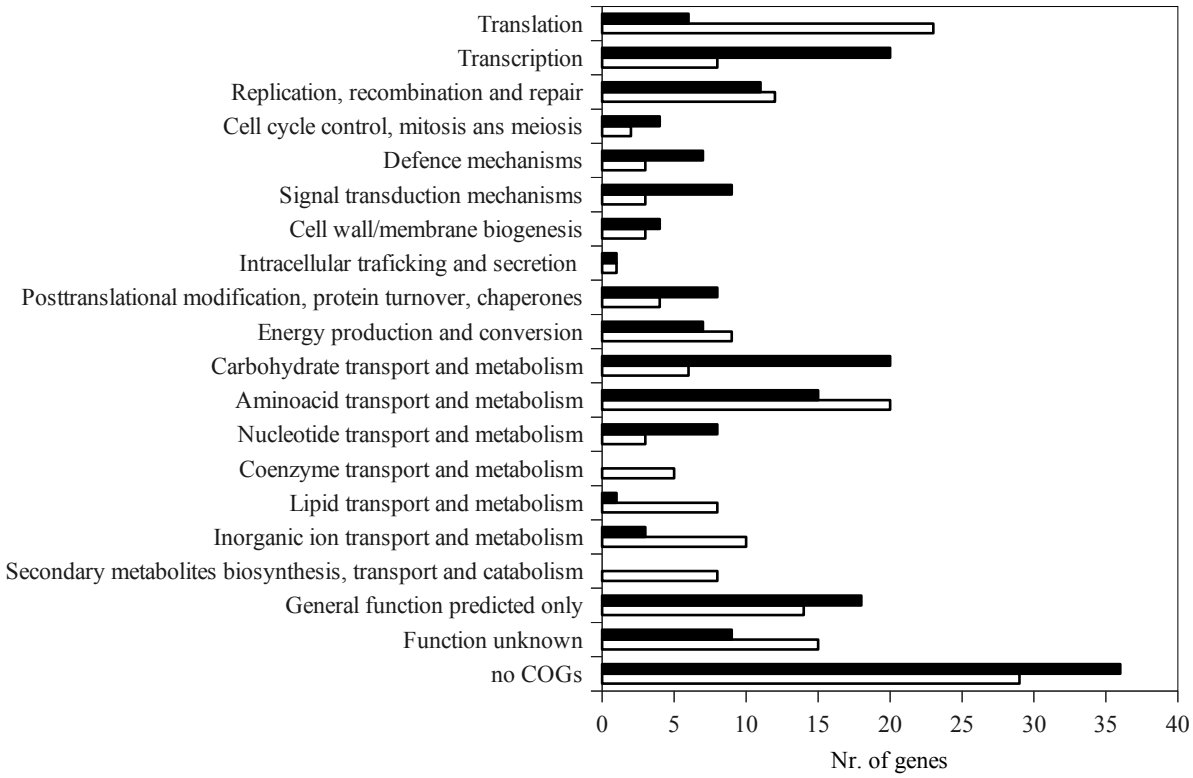

Figure 5. Grouping in clusters of orthologous groups (COGs) based on functional categories of genes differentially expressed in stationary phase biofilms. Black bars: up-regulated genes in biofilm; white bars: down-regulated genes in biofilm. COGs were assigned according to the NCBI database (www.ncbi.nlm.nih.gov).

The microarray results were validated by quantitative RT-PCR. From 12 genes (8 up- and 4 down-regulated genes), 10 genes showed a similar pattern of expression (**Figure 6**).

Up-regulation of carbohydrate metabolism, signal transduction and defence mechanisms in S. mutans biofilms

Carbohydrate metabolism. Among the genes that showed higher expression in biofilms were several genes involved in carbohydrate metabolism: three genes encode enzymes involved in the glycolytic pathway (SMU.99, SMU.985 and SMU.1601), one gene encodes a multiple sugar-binding transporter (SMU.878) and two encode enzymes of two PTS (SMU.115, a fructose-specific IIA component and SMU.1598, a cellobiose IIA component). Biofilms also exhibited differential transcription of several operons involved in the metabolism of carbohydrates, i.e. operons for utilization of lactose and tagatose (SMU.116), galactose (SMU.149), fructose and mannose (SMU.183) and the *msm* operon, encoding uptake and metabolism of multiple sugars.

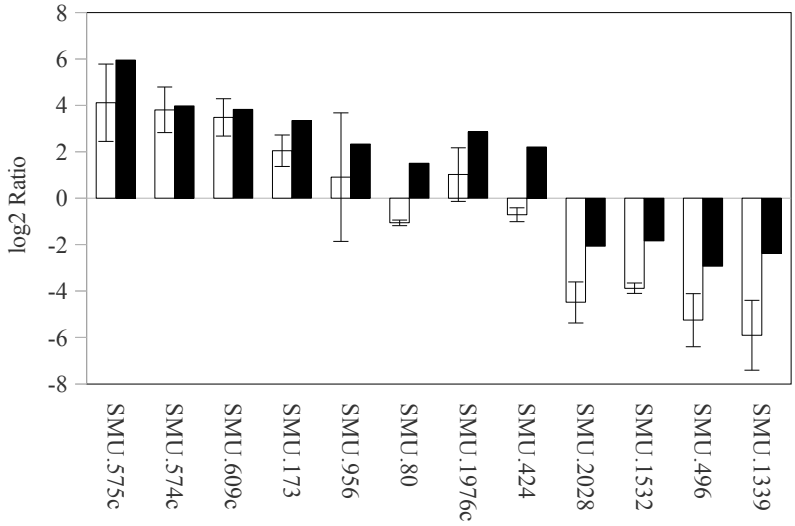

Figure 6. Validation of microarray 2 results with qRT-PCR. Data were generated from two independent RNA preparations, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratios.

Signal transduction mechanisms. The microarray results showed a 62-fold and 16-fold increase in expression of the two genes *lrgA* and *lrgB,* respectively. Both genes are encoding putative membrane proteins. In *Staphylococcus aureus* these genes are involved in cell death and lysis in the stationary phase of growth (Bayles, 2007). The function of this system in *S. mutans* is the topic of Part

II of the Results.

Two toxin-antitoxin (TA) modules, each comprising two genes, were up-regulated in biofilms. The first TA module, consists of SMU.172 - a putative ppGpp-regulated growth inhibitor (mazE – encoding the antitoxin) and SMU.173 - a putative cell growth regulatory protein (mazF – encoding the toxin), and was up-regulated 8.8- and 10.1-fold in biofilms. The second TA module genes, SMU.895 (RelB - a possible DNA-damage-inducible protein) and SMU.896 (RelE – a hypothetical protein) were up-regulated 2.8- and 4-fold. For each pair of genes, the first gene codes for an unstable antitoxin, whereas the second codes for a stable toxin. At homeostasis, there is sufficient antitoxin produced to bind the toxin and blocking its activity. If homeostasis is perturbed by starvation or other type of stress, the toxin is unravelled and the growth is arrested (Magnuson, 2007).

It is generally agreed that toxin-antitoxin pairs play a role in stress response and that they function as ‘‘regulatory switches’’ under conditions of nutrient limitation, permitting the cells to enter a state that confers protection against severe nutrient limitation and possibly other stresses. TA modules may induce bacteriostasis by down-regulation of macromolecular synthesis when nutrients are depleted and reverse the condition when nutrients became available again (Wood, 2009). Indeed, in dental biofilms there are rapid fluctuations in nutrient availability and diffusion.

Down-regulation of translation, energy production, amino acid transport and metabolism in S. mutans biofilms

Translation. An important fraction of down-regulated genes in biofilms were involved in protein synthesis. As many as 13 genes encoding ribosomal proteins from both 50S and 30S ribosomal subunits and a gene encoding a putative translation initiation factor (SMU.2004) were down-regulated in biofilm. This is suggestive of a low level of translation, which is probably due to a slower growth rate of the bacteria in biofilms.

Energy production. Among the genes that were down-regulated in biofilms were those that encode the α- (SMU.1530), β- (SMU.1528), γ- (SMU.1529) and b- (SMU.1532) subunits of F_0F_1 membrane-bound proton-translocating ATPase. F-type ATPases are responsible for pumping H^+ out of the cytoplasm and keeping the intracellular pH suitable for cell metabolism (Thedei *et al.*, 2008) and for generating energy by coupling H^+ import with synthesis of ATP.

Amino acid transport and metabolism. Several genes from this category were also down-regulated in *S. mutans* biofilms. Two genes, *oppC* and *oppD*, encoding a putative ABC transporter of

oligopeptides (Higgins, 2001), were repressed. The genes SMU.933, SMU.934 and SMU.936, encoding another putative ABC transporter with unknown specificity, were down-regulated 4.03-, 4.46- and 2.47, respectively.

Hypothetical proteins, poorly characterized proteins and proteins not matching COGs. An important fraction of the differentially expressed genes code for proteins with unknown functions or for poorly characterized ORFs. Others have no corresponding homolog in COGs. Among these, SMU.609, a 40 kDa surface protein was up-regulated 10-fold in biofilms. It has been shown that a deletion in the C-terminus of this protein affected murein hydrolase activity (Catt & Gregory, 2005). Murein hydrolases are involved in breaking peptidoglycan and thus in remodelling of the cell-wall. However, a mutant lacking SMU.609 showed no difference with the wild type with respect to murein hydrolases activity or biofilm formation (data not shown).

Among the down-regulated genes were also SMU.2028 (4.0-fold), encoding the β-D-fructosyltransferase Ftf, and SMU.1396 (2.5-fold), encoding glucan binding protein GbpC. The Ftf protein, located outside of the cell, catalyzes the cleavage of sucrose to synthesize extracellular fructan polymers. Fructans serve as extracellular storage compounds that can be metabolized during periods of nutrient starvation (Burne *et al.*, 1996). Surface-associated glucan-binding proteins (Gbp) adhere to glucans, promoting plaque formation (Mattos-Graner *et al.*, 2001).

Genes encoding bacteriocin production and bacteriocin immunity were down-regulated in biofilms between 2.2- and 2.9-fold. Other down-regulated genes are located on TnSmu2 (SMU.1333 to SMU.1345). Bioinformatic analysis suggests that TnSmu2 encodes a system for polyketide synthesis.

3.1.3 Gene expression in exponentially grown biofilms in the presence of sucrose (Microarray 3)

To assess the influence of sucrose on gene expression in biofilms, the transcriptome profiles of *S. mutans* grown in biofilm in medium supplemented with sucrose and in medium with glucose, both in exponential and stationary phases of growth, were compared.

After data normalization and filtering, 23 genes were found to be up-regulated and 6 genes were found to be down-regulated in the exponential biofilm grown in medium supplemented with sucrose (**Table 3**). Quantitative RT-PCR was performed for a set of genes (n=10) selected from the microarray experiment. 8 out of 10 genes showed similar expression with real-time PCR, thereby confirming the microarray results (**Figure 7**). A comparison of gene expression ratios between microarray and real-time PCR showed a very good correlation (R^2=0.8) (**Figure 8**).

Upon addition of sucrose, the sucrose-specific phosphotransferase-PTS system, the major sugar transport system, was induced. The EII^{Suc} locus comprises 3 genes, SMU.1841 (*scrA*; 10.3-fold up-regulated), the protein of which converts sucrose into sucrose-6-phosphate, SMU.1843 (*scrB*; 2.4-fold up-regulated), encoding sucrose-6-phosphate hydrolase, which cleaves sucrose-6-phosphate to glucose-6-phosphate and fructose and SMU.1844 (*scrR*; 2-fold up-regulated), encoding the sucrose operon repressor (Wang & Kuramitsu, 2003).

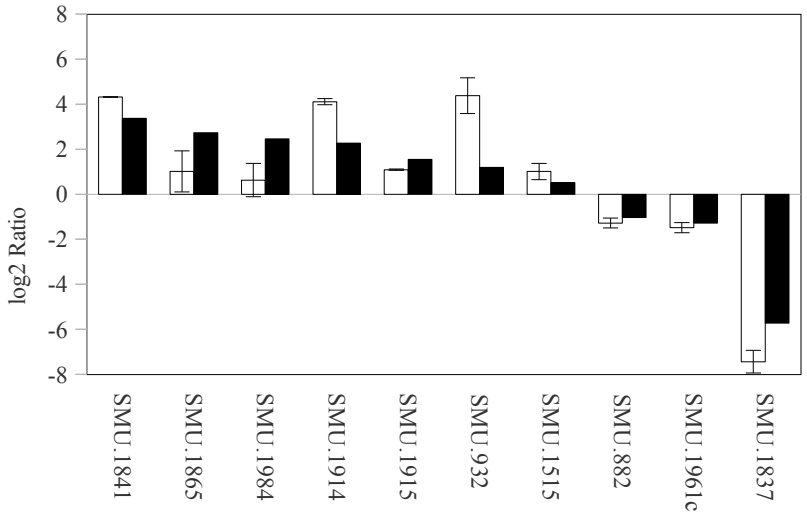

Figure 7. Validation of microarray 3 results with qRT-PCR. Data are generated from two independent RNAs, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratio

Another group of up-regulated genes were those that encode bacteriocins and bacteriocin immunity-like proteins. Bacteriocins are ribosomally synthesized peptides with antimicrobial activity (Rogers, 1976), which may be required by *S. mutans* for successful colonization and survival in dental plaque. Bacteriocins are active towards a broad spectrum of streptococci. Organisms are resistant to self-produced bacteriocins through the action of immunity proteins (Dirix *et al.*, 2004). As many as 12 putative bacteriocins genes were up-regulated between 8.3- and 3.4-fold in *S. mutans* biofilms in the presence of sucrose after 4 hours of growth. Some of the genes required for competence were also up-regulated (SMU.1980c and SMU.1984). SMU.1837, encoding phospho-2-dehydro-3-deoxyheptonate aldolase (DAPH), was strongly down-regulated in sucrose biofilms. DAPH catalyses the first step in the synthesis of aromatic amino acids and vitamins. The synthesis of aromatic amino acids, especially L-tryptophan, represents a high energy investment for the cell (Mortlock, 1992) and the bacteria grown in high density in biofilms in sucrose apparently down-regulate DAPH to maximize efficiency of

energy consumption.

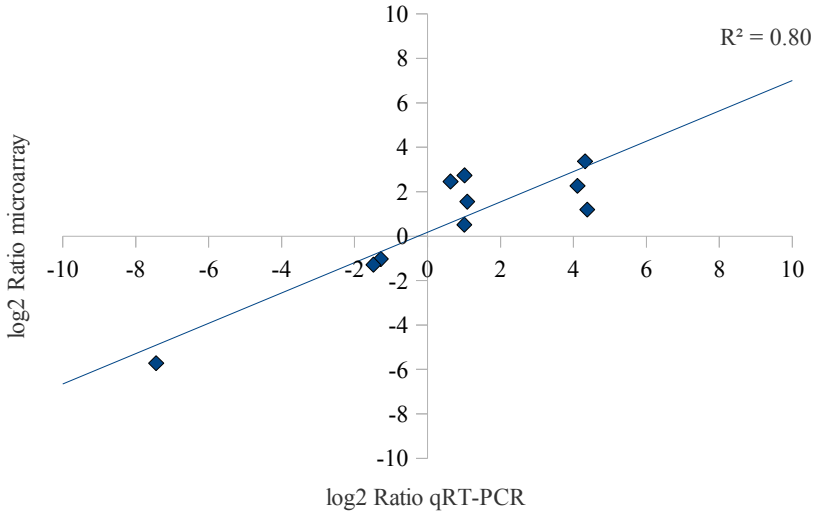

Figure 8. Correlation of gene expression between microarray 3 and qRT-PCR. Each point represents one gene. Fold changes in gene expression after comparison of biofilms grown with glucose *vs*. sucrose in exponential phase are plotted.

3.1.4 Gene expression in stationary phase biofilms in the presence of sucrose (Microarray 4)

Microarray data analysis revealed many genes being differential expressed in response to sucrose in stationary phase biofilms compared with biofilms in the exponential growth phase. 78 genes were up-regulated and 64 genes were down-regulated (**Table 2** of the **appendix**). To validate the microarray results, real-time PCR was performed on a set of 10 genes (five genes being down-regulated and five up-regulated in microarray). The differential expression of nine out of ten genes was confirmed by real-time PCR (**Figure 10**). They showed the same trend with both methods with a very good correlation (R^2=0.95) (**Figure 11**).

Genes were grouped in functional categories (**Figure 9**). In stationary phase sucrose-grown biofilms, genes encoding DNA recombination and repair functions were up-regulated. A putative site-specific DNA methylase as well as an adenine methylase were up-regulated 5.2- and 3.6-fold respectively. An ATPase involved in DNA repair was up-regulated 3.7–fold. In addition, genes encoding enzymes involved in initiation and execution of chromosome replication (*dnaA, dnaB* and *dnaN*) were expressed at a higher level in sucrose-grown biofilms. This could indicate that there is

more DNA damage in sucrose-grown biofilms and that bacteria cope with it by activation of repair enzymes.

Apart from being involved in the uptake of sugars, PTS have a role in the regulation of a variety of processes required for cell growth in biofilms and for cell maintenance (Rathsam *et al.*, 2005a). Since the medium used for this experiment was THYE, a rich medium containing a low amount of glucose, it could be observed that upon addition of sucrose, the 16h-old biofilms exhibited a repression of genes involved in sugar uptake. This may be due to catabolite repression. In turn the bacteria are protecting themselves by preventing further uptake of sugars which, by avoiding more acid production and acidification of the cytoplasm, allows them to maintain an optimal pH inside the cells. Shemesh *et al.,* (2007b) showed that also several genes involved in *S. mutans* biofilm formation in THYE medium supplemented either with 1% sucrose and 0.2% glucose, or with 4% sucrose and 0.2%

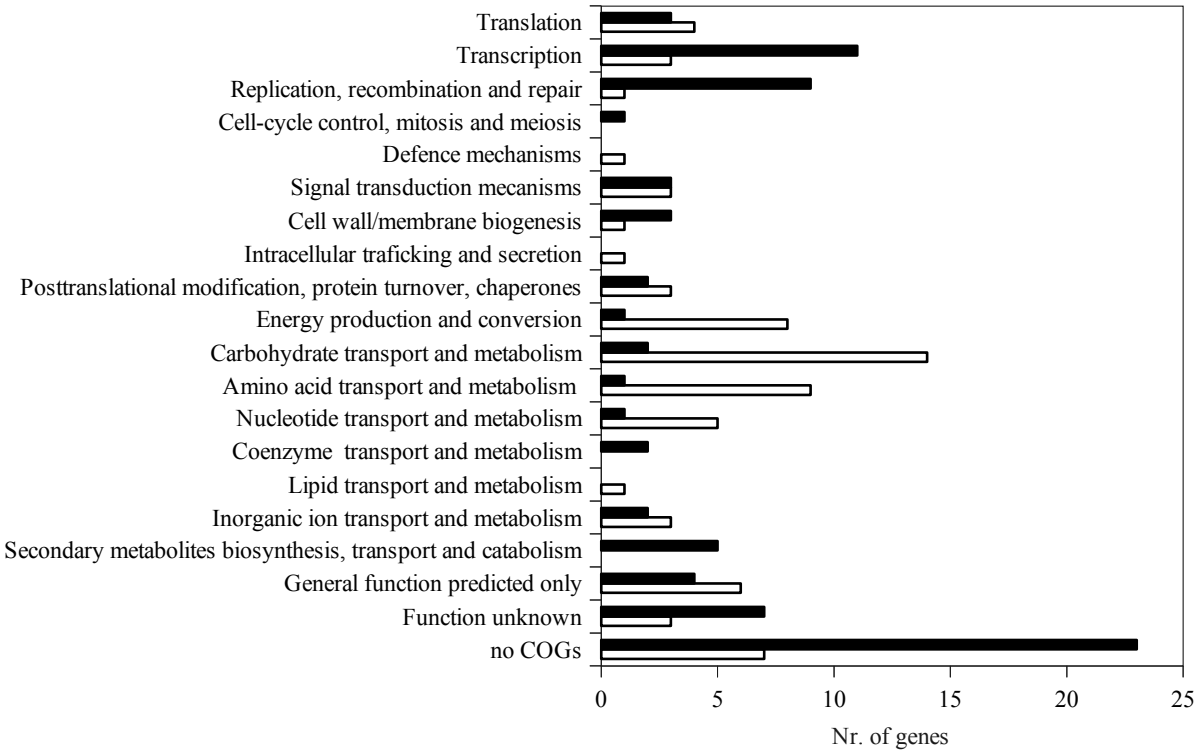

Figure 9. Differentially expressed genes in microarray experiment 4 grouped in clusters of orthologous groups functional categories. Black bars: up-regulated genes in biofilm grown with sucrose; white bars: down-regulated genes in biofilm grown with sucrose.

glucose were down-regulated when compared with 0.2% glucose-containing THYE alone.

Genes involved in amino acid transport and energy conversion were also down-regulated in sucrose-grown biofilms.

Overall, this down-regulation of metabolic processes suggests a decreased bacterial growth rate in stationary phase when biofilm is formed in the presence of sucrose.

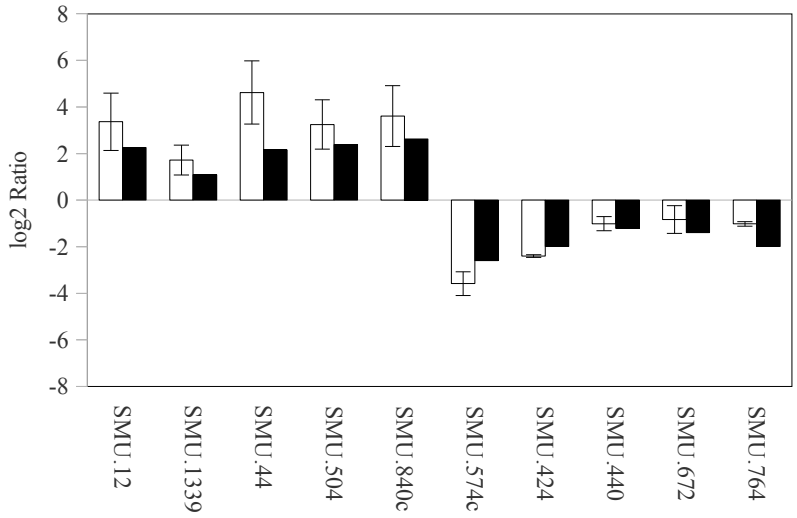

Figure 10. Validation of microarray 4 results with qRT-PCR. Data are generated from two independent RNA preparations, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratios.

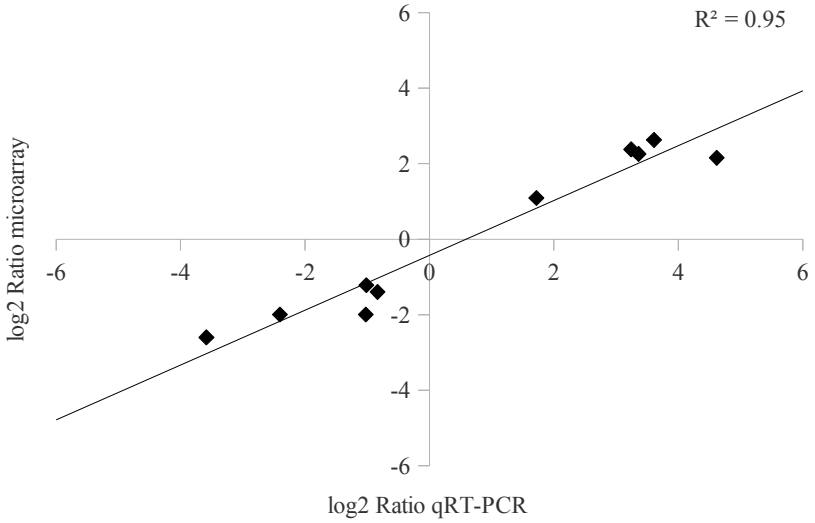

Figure 11. Correlation between gene expression in *S. mutans* biofilms studied by microarray 4 and qRT-PCR. Each point represents a gene. Fold changes in gene expression after comparison of biofilms grown with glucose *vs*. sucrose in exponential phase are plotted.

3.1.5 Gene expression in starved and fed biofilms

The goal of these experiments was to gain insight in the global changes in *S. mutans* gene expression when going from a medium that is poor in nutrients (saliva) to one rich in nutrients (semi-defined medium supplemented with 0.15% glucose and 0.15% sucrose), and the reverse situation when returning from a nutrient-rich medium to saliva (**Figure 12**). This is of interest, since in real life, during most of the time a limited supply of nutrients is available in the oral cavity (saliva only). During times of food consumption though, the availability of nutrients is greatly increased.

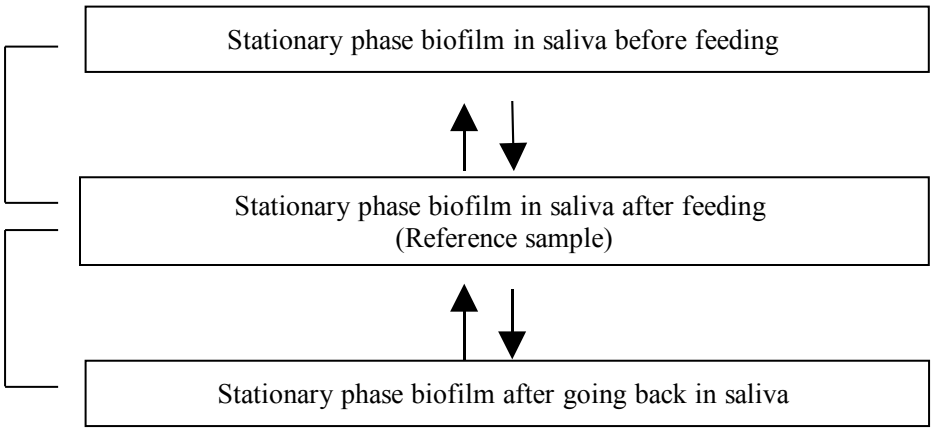

Figure 12. Design of the microarray experiments.

3.1.5.1 Starving stationary phase biofilm *vs*. fed stationary phase biofilm (microarray experiment 5)

Gene expression profiles were compared between 64.5 hours old biofilms before and after feedings. Upon addition of fresh medium to a 16 hours-starved biofilm, a major change in the gene expression profile was observed. The expression of 227 genes was increased, whereas 155 genes were down-regulated **(Table 3 of the Appendix).** For seven out of ten genes chosen, quantitative reverse-transcriptase PCR confirmed the microarray experiments (**Figure 14**). There appeared to be a bias in that results from microarray analysis showed higher ratios than results from qRT-PCR. This might be due to technical variability of the microarray technology.

To assess the possible functions of the differentially regulated genes, they were grouped in functional categories (**Figure 13**). The functional category containing the highest number of up-regulated genes was translation, with about 30 genes (20 % from the total genes of this category). This category was followed by transcription (22 genes) and inorganic ion transport and metabolism (13

genes).

19 genes encoding ribosomal proteins, which are part of the protein synthesis machinery, were up-regulated. Translation initiation factors IF-1 and IF-3, as well as elongation factors P and G were induced 6.8- and 3.9 fold, and 2.4- and 2.3-fold respectively.

An important number of genes encoding functions in transcriptional processes were up-regulated. Among them was the gene for RNA polymerase (*rpoA*) which showed a 9.7-fold increase. As many as 13 putative transcriptional regulators were altered in expression. For example, SMU.1168 (a putative transcriptional regulator) was highly induced (14.7 - fold) upon feeding. This gene is located upstream and in the pposite direction from a cluster containing genes coding for ABC transporters. The *brpA* gene, predicted to encode a surface-associated protein involved in biofilm formation, acid tolerance and cell division (Wen *et al.*, 2006) was up-regulated 2.91-fold. Another putative response regulator-encoding gene, *bglC*, was up-regulated 2.3-fold. This gene is located in the operon responsible for the uptake of

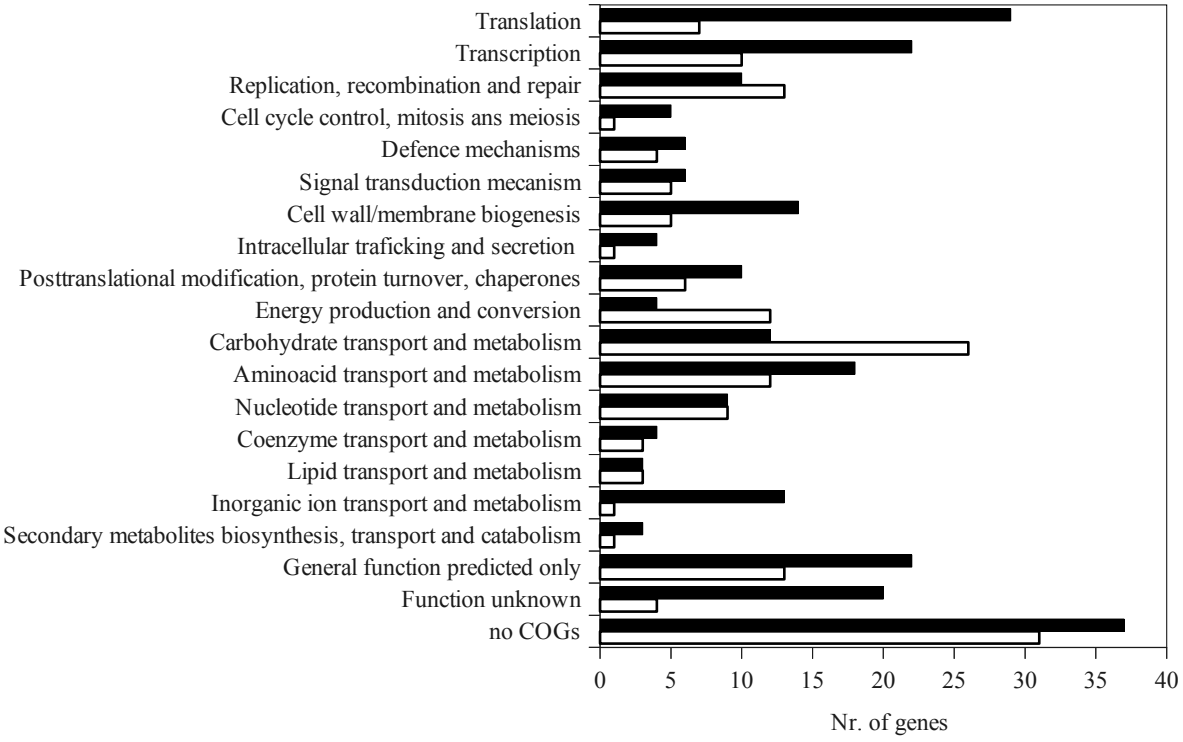

Figure 13. Differentially expressed genes in microarray experiment 5 grouped in clusters of orthologous groups functional categories. Black bars: up-regulated genes in fed biofilms; white bars: down-regulated genes in fed biofilms.

β-glucosides. In *Streptococcus gordonii*, a mutant in *bglC* gene is defective in growth rate and in vitro biofilm formation (Kilic *et al.*, 2004).

Genes involved in the uptake of ions (SMU.1709; putative potassium uptake protein) and transport of ions (SMU.183, putative Mn/Zn ABC transporter; SMU.182, iron/manganese ABC transporter; SMU.1852, putative magnesium/cobalt transport protein; SMU.770c, putative manganese transporter; SMU.1563, putative cation-transporting ATPase) were expressed to a higher extent in fed biofilms. Gene SMU.1841, encoding a putative sucrose-specific IIABC component (ScrA) was induced 4.7 fold. This is the major direct sucrose uptake protein in *S. mutans*. SMU.307 (glucose-6-phosphate isomerase), SMU.1191 (6-phospofructokinase) and SMU.596 (phosphoglyceromutase) were also up-regulated more than 2-fold. These genes encode all for enzymes involved in glycolysis pathway/sucrose and starch metabolism. Relatively fewer genes showed decreased transcription upon addition of nutrient-rich fresh medium. Among these were genes involved in replication, recombination and repair, energy production and carbohydrate metabolism. Taking into account that bacteria were in saliva as the sole food source for a long time, the metabolic processes were reduced, together with

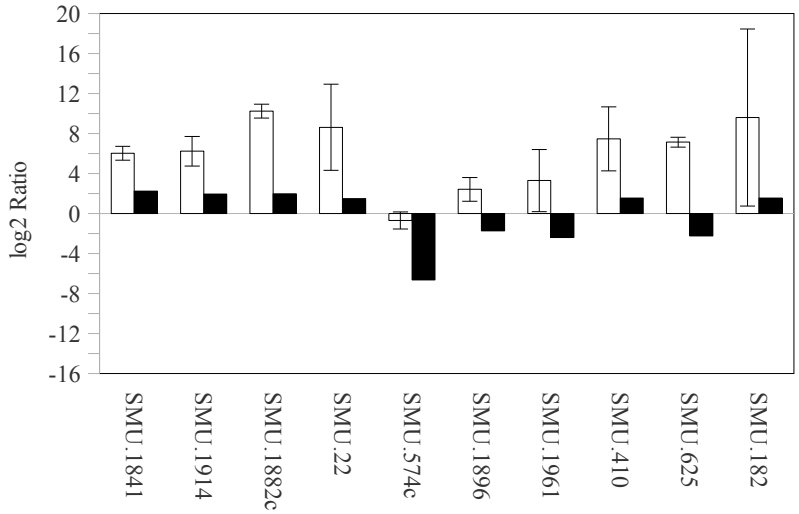

Figure 14. Confirmation of microarray 5 results with qRT-PCR. Data were generated from two independent RNA preparations, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratios.

energy production and replication. It has been shown that, at least for some laboratory strains and clinical isolates of *Streptococcus mutans,* glucose and sucrose uptake is induced by starvation (Lodge & Jacobson, 1988). In our microarray experiments with the UA159 strain, several PTS were constantly

induced in starving bacteria. Furthermore, after feeding, only specific PTS responsible for uptake of the existing sugar (here for sucrose) in the medium are increased.

3.1.5.2 Stationary phase fed biofilms *vs.* stationary phase restarved biofilms (microarray experiment 6).

In experiment 6, the gene expression profile of fed biofilm cultures was compared with that of biofilms, which were re-starved by keeping the bacteria for 45 minutes in saliva. The same labelled cDNA obtained from fed biofilms was used as a reference for hybridization in both experiments 5 and 6. All nine genes chosen for validation by quantitative reverse-transcriptase PCR showed the same pattern of expression with a very good correlation, thereby confirming the microarray results (**Figures 16 and 17**). The category encompassing the highest number of repressed genes was translation (49 genes, 34% from the total in this category), followed by transcription (22 genes, 13% from the total). About 47 genes involved in carbohydrate transport and metabolism were induced in starving bacteria (1/3 from the total genes of this category), (**Figure 15**). Among these genes, there were some constituents of a several PTS which are responsible for sugar uptake in *S. mutans*. To a lesser

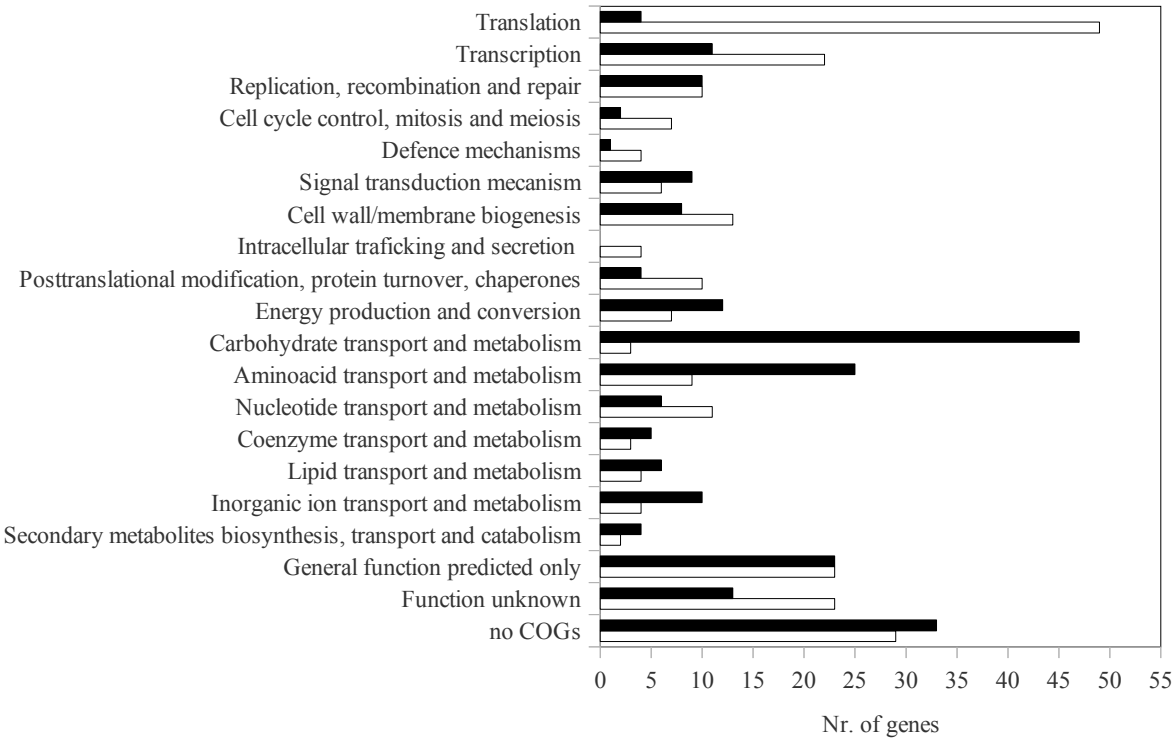

Figure 15. Differentially expressed genes in microarray experiment 6 grouped in clusters of orthologous groups functional categories. Black bars: up-regulated genes in restarved biofilms; white bars: down-regulated genes in restarved biofilms.

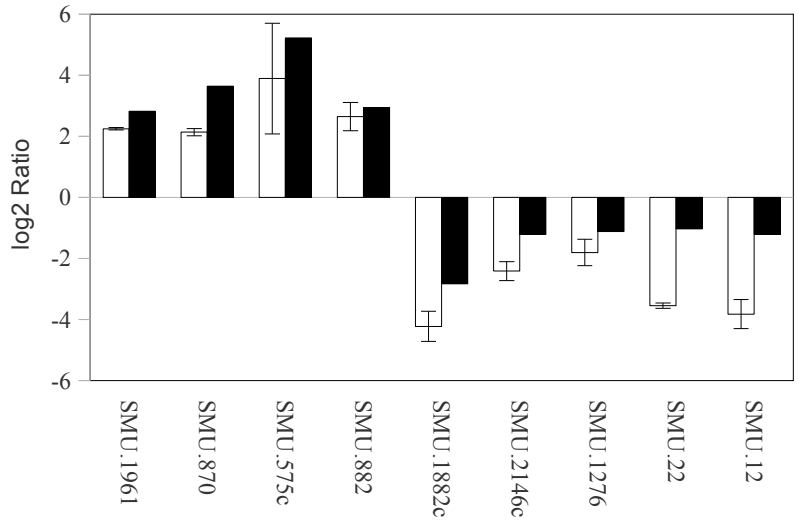

Figure 16. Confirmation of microarray 6 results with qRT-PCR. Data are generated from two independent RNAs, each in triplicate. White bars: qRT-PCR ratios; Black bars: microarray ratios.

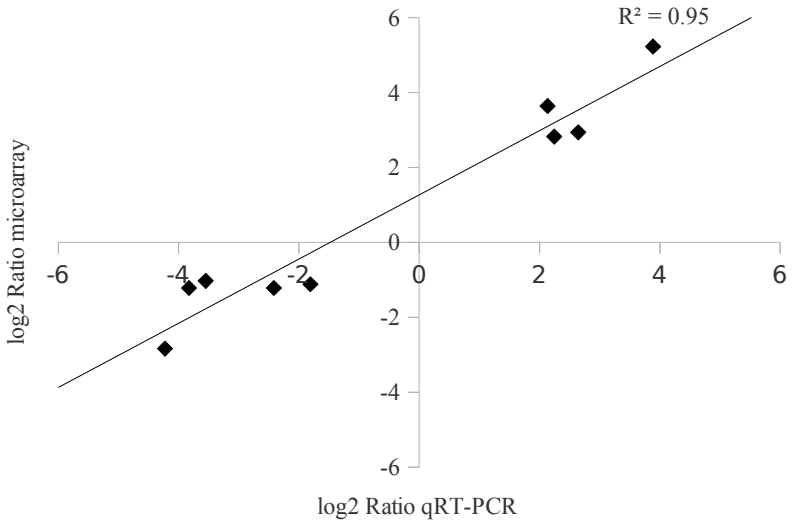

Figure 17. Correlation of gene expression between microarray 6 and qRT-PCR. The points represent genes tested by both methods. Fold changes in gene expression after comparison of fed *vs*. restarved biofilms are plotted. R^2 indicates linear regression.

extent, some of these PTS were also expressed in experiment 5 in bacteria starving for a longer period of time. Apparently, *S. mutans* increases expression of sugar up-take systems under condition of sugar limitation. Genes involved in the metabolism of the amino acid histidine were also up-regulated in starved biofilms.

Almost all of the genes associated with ribosomal protein synthesis, elongation- and translation initiation factors were repressed again upon starvation, therefore reducing protein synthesis. DNA polymerase and RNA polymerase were also repressed 2.8-and 9.5-fold.

When comparing experiment 5 and 6, 40 % from the total number of up-regulated genes which showed increased expression after feeding in experiment 5 (97 genes) were found to be repressed 45 minutes after the bacteria were transferred in saliva.

All these findings indicate that *S. mutans* modulates gene expression in response to environmental conditions and nutrient availability**.**

3.1.6 General discussion

This study reports global gene expression profiles of *S. mutans* when grown under different biofilm conditions. Starting with the premise that bacteria grown in biofilms have a different phenotype than planktonically-grown bacteria, transcriptome changes of *S. mutans* were assessed upon growth in planktonic versus biofilm conditions. Apparently, there are no striking differences between the transcriptome of biofilm cultures and planktonic cultures of *S. mutans* during the exponential phase of growth. The up-regulation of the transport system for manganese could indicate that this component is required for biofilm maintenance.

The differences in gene expression between biofilm-grown cells and planktonically grown cells were much larger in the stationary phase. Numerous up-regulated genes are likely to be involved in transcription and among those there were several transcriptional regulators which may play a role in regulation of biofilm growth. Several sugar transporters were up-regulated in biofilms. It was shown previously that in *S. mutans* the relative rate of synthesis of enzymes involved in sugar catabolism was enhanced during the initial stage of biofilm formation (2 hours from biofilm establishment) (Welin *et al.*, 2004). The synthesis of these enzymes was diminished in a 3-day old biofilm grown in chemostat (Svensäter, *et al.* 2001). Protein synthesis was down-regulated in stationary phase biofilms, indicating a slower growth. This is also in agreement with the down-regulation of the ABC transporters, which are responsible for the translocation of a large variety of molecules across the membrane (amino-acids,

sugars, ions**)** (Ajdić *et al.*, 2002). Shemesh *et al.,* (2007b) observed a similar down-regulation of protein synthesis and transport systems and hypothezised that in biofilms bacteria have a limited but more specific metabolic activity. The down-regulation of the F_0F_1 membrane-bound proton-translocating ATPase, which is responsible for pumping H^+ out of the cytoplasm and keeping the intracellular pH suitable for cell metabolism (Thedei *et al.*, 2008), indicates that there is enough ATP in the cells to drive hydrogen ions out of the cell, thus maintaining cellular pH homeostasis (McNeill & Hamilton, 2004). Similar transcriptome changes have been observed with microarray studies in *Porphyromonas gingivalis* biofilms (Lo *et al.*, 2009).

Several putative bacteriocin genes and one gene encoding a putative immunity protein gene were repressed in biofilms. This finding seems to be in conflict with what was found by Shemesh *et al.,* (2007b) in a similar experiment, where bacteriocin genes were found to be up-regulated in stationary phase biofilms. Differences in experimental conditions like medium and growth in aerobic conditions could account for this discrepancy.

Sugar availability and type have an important influence on *S. mutans* biofilm formation (Burne *et al.*, 1997; Shemesh *et al.*, 2007a). In the presence of sucrose, the bacterium is able to form an adhesive, sticky biofilm, which allows it to persist on the tooth surface. This is due to the action of glycosyltransferases and glucan binding proteins, which contribute to the formation of extrapolysaccharide matrix that holds the biofilm together. These proteins are well known for their role as virulence factors (Kuramitsu, 1993). If the only carbon source is glucose, the biofilm produced by *S. mutans* is thinner and loosely bound with an increased chance of being detached.

Addition of sucrose to both an exponential and a stationary phase biofilm changed the gene expression profile. The *scrA* gene, which encodes the enzyme II component of the PTS for sucrose was the most highly up-regulated gene, indicating that this is the main system for sucrose uptake in *S. mutans.* Bacteriocin genes were expressed at a higher level in the exponential phase of growth in the presence of sucrose. Since it was shown previously that the bacteriocin genes are highly expressed in stationary phase (van der Ploeg, 2005), it can be hypothesized that addition of sucrose has an indirect effect on bacteriocin-gene expression through increased cell density and biofilm thickness. *S. mutans* produces bacteriocins to compete with other streptococcal species in the oral biofilm environment (Merritt *et al.*, 2005a). Furthermore, the *comC* gene was also up-regulated in sucrose-containing exponentially phase biofilms. ComC, together with the two-component signal transduction system ComDE, regulates competence, bacteriocin production and biofilm formation in *S. mutans* (Li *et al.,*

2002b, van der Ploeg 2005). Stationary phase sucrose-grown biofilms showed an increased expression of genes involved in DNA replication, recombination and repair. Perhaps at this stage of growth cells are still dividing, but probably they cope with environmental stresses that affects to a certain extent cell division. Hahn et al. proposed that up-regulation of DNA repair enzymes is essential for adaptation of *S. mutans* to low pH. Indeed, the pH of biofilms grown with sucrose was lower than that of biofilms grown with glucose (Hahn *et al.*, 1999). In contrast with biofilms grown with glucose as the carbon source, the biofilms grown with sucrose showed a decreased expression of genes encoding carbohydrate metabolism. It has been shown that exposure of *S. mutans* to high amounts of sugars represses sugar transporters to prevent damage by acidification of the cytoplasm due to carbohydrate metabolism (Lemos *et al.*, 2005).

It was found in this study that nutrient deprivation greatly changed the gene expression pattern of *S. mutans*. The transition from starvation to feeding conditions in biofilms induced changes in the transcriptome, which are likely to have a profound effect on the metabolism. Upon feeding, translation and transcription increased, suggesting that *S. mutans* produces new proteins for metabolic processes. Expression of genes encoding the uptake of ions (Zn^{2+}, Mn^{2+} and Fe^{2+}) also increased as well as expression of those associated with cell wall metabolism and membrane transport. Increased gene expression for manganese uptake was also observed in exponentially-growing biofilms. The functional category containing genes that were repressed upon feeding was carbohydrate metabolism. The same pattern has been seen in biofilms grown in the presence of higher amounts of sucrose. This suggests a slower rate of uptake of sugars to prevent a fast acidification of the cytoplasm, thus keeping a uniform pH within the biofilm. Since changes in gene expression are rather rapid, bacteria are able to take up nutrients within minutes after they became available. Also, the metabolic activity seems to be lower in biofilms lacking nutrients. Using a proteomic approach to identify proteins expressed in mature biofilms of *S. mutans* using a continuous system, Rathsam *et al.,* (2005b) showed a down-regulation in protein expression. Among the down-regulated proteins in biofilms were those associated with sugar metabolism, amino acid transport, translation, and fatty acids biosynthesis. A similar pattern was observed in this work in biofilms depleted of nutrients for about 16 hours, but at the level of transcription.

When *S. mutans* in biofilms returned from feeding conditions to starvation the reverse situation was observed, even though the starvation period was only 45 minutes compared with about 16 hours starvation before the feeding. It seems important for survival of *S. mutans* that the stress response is

turned on soon after the onset of starvation. This was also observed in *Enterococcus faecalis* (Hartke *et al.*, 1998) and *Listeria monocytogenes* (Herbert & Foster, 2001). In our experiment, genes involved in sugar metabolism were up-regulated even after a short period of starvation. Translation was the most down-regulated category (34% from the total number of genes in this category) followed by transcription, indicating that once they are nutrient-deprived, *S. mutans* severely decreases its protein synthesis. A general pattern was observed when there was an alternation of periods of nutrient availability and nutrient deprivation. It was found that about 100 genes, which were expressed under nutrient-replete conditions, were repressed even after a short period of starvation and remained repressed until the next feeding period.

Overall, these data indicate that when *S. mutans* grows in biofilms, the organism increases its chances for survival by decreasing metabolic processes to reduce energy consumption and at the same time raises the expression of virulence factors for its persistence.

Table 2. Differentially expressed genes in biofilms *vs.* planktonically grown cells in exponential phase (p-values<0.05). Ratios represent the value for each differentially expressed gene in biofilms.

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.183	putative Mn/Zn ABC transporter	sloB	3.43	3.50E-02
SMU.182	putative ABC transporter, ATP-binding protein; possible iron and/or manganese ABC transport system	sloA	3.41	4.46E-02
SMU.1474c	ribonuclease Z		3.17	2.14E-02
SMU.1496	galactose-6-phosphate isomerase, subunit LacA	lacA	2.79	2.78E-02
SMU.1263	putative phosphoribosyl-ATP pyrophosphatase / phosphoribosyl-AMP cyclohydrolase	hisI	2.64	4.16E-02
SMU.184	putative ABC transporter, metal binding lipoprotein; surface adhesin precursor saliva-binding protein; lipoprotein receptor LraI (LraI family)	sloC	2.63	4.27E-02
SMU.496	putative cysteine synthetase A; O-acetylserine lyase	cysK	2.42	5.16E-03
SMU.770c	putative manganese transporter		2.16	2.81E-02
SMU.1187	glucosamine-fructose-6-phosphate aminotransferase	glmS	2.10	2.61E-02
SMU.1491	PTS system, lactose-specific enzyme IIBC EIIBC-LAC	lacE	2.05	1.96E-02
SMU.460	putative amino acid ABC transporter, permease		2.04	3.43E-03
SMU.1515	hypothetical protein CovX (VicX)	covX	2.03	4.48E-02
	down-regulated genes			
SMU.1117	NADH oxidase (H2O-forming)	naoX	0.20	2.13E-02
SMU.629	putative manganese-type superoxide dismutase, Fe/Mn-SOD	sod	0.27	3.46E-02
SMU.1764c	hypothetical protein		0.30	3.60E-02
SMU.1760c	hypothetical protein		0.31	2.45E-02
SMU.1737	putative 3-hydroxymyristoyl-(acyl carrier protein) dehydratase	fabZ	0.32	2.99E-02
SMU.1762c	hypothetical protein		0.33	2.10E-02
SMU.1782	hypothetical protein		0.34	1.93E-02
SMU.764	alkyl hydroperoxide reductase	ahpC	0.34	3.03E-02
SMU.1774c	hypothetical protein		0.36	1.20E-02
SMU.1784c	putative Eep protein homolog; possible membrane-associated Zn-dependent proteases		0.36	1.94E-02
SMU.1757c	hypothetical protein		0.37	2.66E-02
SMU.1754c	hypothetical protein		0.38	2.43E-02
SMU.127	putative acetoin dehydrogenase (TPP-dependent), E1 component alpha subunit	adhA	0.38	1.09E-02
SMU.1116c	hypothetical protein		0.39	3.04E-02
SMU.1735	putative acetyl-CoA carboxylase beta subunit	accD	0.40	1.89E-02
SMU.205c	hypothetical protein		0.41	1.21E-02
SMU.206c	hypothetical protein		0.42	2.16E-02
SMU.1004	glucosyltransferase-I	gtfB	0.42	2.09E-03
SMU.1416c	putative mutator protein	mutT	0.43	3.16E-02
SMU.610	cell surface antigen	spaP	0.44	4.35E-03
SMU.202c	hypothetical protein		0.45	2.90E-02
SMU.1750c	hypothetical protein		0.45	3.58E-02
SMU.201c	putative transposon protein		0.47	7.20E-03
SMU.1988c	putative DNA binding protein		0.47	1.31E-02
SMU.198c	putative conjugative transposon protein		0.47	1.52E-02
SMU.1504c	hypothetical protein		0.49	4.84E-04

Table 3. Differentially expressed genes in biofilms grown in the presence of sucrose *vs.* biofilms grown in the presence of glucose (p-values<0.05). Ratios represent the value for each differentially expressed gene in biofilms grown in the presence of sucrose.

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.1841	putative PTS system, sucrose-specific IIABC component	scrA	10.32	3.40E-06
SMU.1908c	bacteriocin production		8.33	2.70E-02
SMU.423	bacteriocin production		7.97	1.70E-02
SMU.152	bacteriocin production		7.63	1.51E-02
SMU.1903c	bacteriocin production		7.62	3.05E-02
SMU.150	bacteriocin production	nlmA	7.54	2.24E-02
SMU.1906c	bacteriocin production		7.51	2.92E-02
SMU.151	bacteriocin production	nlmB	7.34	1.88E-02
SMU.1865	putative A/G-specific DNA glycosylase	mutY	6.67	5.75E-03
SMU.1904c	bacteriocin production		5.95	3.28E-02
SMU.1984	putative competence protein	comYC	5.51	3.99E-02
SMU.1913c	putative immunity protein, BLpL-like		5.44	1.99E-02
SMU.1910c	bacteriocin production		5.26	1.42E-02
SMU.1914c	bacteriocin production	bsmA	4.81	2.60E-02
SMU.1905c	bacteriocin production		4.73	1.45E-02
SMU.1909c	bacteriocin production		4.70	2.21E-02
SMU.1902c	bacteriocin production		3.40	4.47E-02
SMU.1980c	conserved hypothetical protein		2.95	1.63E-02
SMU.1915	competence stimulating peptide, precursor	comC	2.94	9.91E-03
SMU.1843	sucrose-6-phosphate hydrolase	scrB	2.39	1.56E-02
SMU.932	hypothetical protein		2.29	9.68E-03
SMU.933	putative amino acid ABC transporter, periplasmic amino acid-binding protein		2.18	2.21E-03
SMU.1844	sucrose operon repressor	scrR	2.06	7.05E-07
	down-regulated genes			
SMU.882	multiple sugar-binding ABC transporter, ATP-binding protein	msmK	0.50	2.12E-02
SMU.2040	putative transcriptional regulator; repressor of the trehalose operon	treR	0.49	5.91E-03
SMU.1564	putative glyen phosphorylase	glgP	0.49	9.78E-06
SMU.1961c	putative PTS system, sugar-specific enzyme IIA component		0.41	8.53E-03
SMU.1956c	hypothetical protein		0.38	5.95E-05
SMU.1837	phospho-2-dehydro-3-deoxyheptonate aldolase	aroH	0.02	1.70E-07

Part II. Involvement of holin-like proteins encoded by *lrgAB* and *cidAB* in stationary phase survival

3.2.1 General aspects

The transcriptome analysis of *S. mutans* biofilms (Part I of the Results) showed that the *lrgA* and *lrgB* genes were highly up-regulated in stationary phase biofilm cultures compared with stationary phase planktonic cultures (**Table 4**). Both genes were down-regulated in stationary phase biofilm cultures grown with sucrose when compared to stationary phase biofilm cultures grown without sucrose. The *lrgAB* genes were also up-regulated after transition from nutrient-replete medium to saliva, which is poor in nutrients.

In the genome data base of *S. mutans* (http://www.genome.jp/kegg/), the two genes are annotated as encoding holin-like proteins with putative functions. Holins are small membrane proteins that control the precise timing of lysis during release of bacteriophage progeny after infection (Wang *et al.*, 2000). Holins function at the post-transcriptional level to control the access of murein hydrolases to the cell-wall, either by mediating the release and activation of membrane associated murein hydrolases or by controlling the transport of murein hydrolase across the membrane (Park *et al.*, 2006). Bacteriophage antiholins are homologous to holins but contain two or three additional amino acids at the N-terminus and counteract the action of holins.

Table 4: Differential expression of the *lrg* operon

Condition	Fold change *lrgA*	Fold change *lrgB*
Planktonic *vs.* biofilm (stationary phase)	62	15
Biofilm in glucose *vs.* biofilm in sucrose (stationary phase)	-20	-6
Starved *vs.* fed biofilms	-50	-100
Fed *vs.* restarved biofilms	37	19

Recent studies have shown that in *S. aureus* the *lrgAB* genes influence cell lysis. Both genes are thought to encode for antiholin-like membrane proteins that inhibit the formation of channels for transport of murein hydrolase. These channels, assumed to be encoded by *cidAB*, are thought to function like holins. An *lrgAB* mutant of *S. aureus* exhibited increased extracellular murein hydrolase activity and enhanced penicillin-induced killing when approaching stationary phase (Groicher *et al.*, 2000). In contrast, in a *cidA* mutant of *S. aureus*, extracellular murein hydrolase activity was almost

abolished (Rice *et al.*, 2007). The expression of these genes is controlled by transcriptional regulators, including the two-component system encoded by *lytRS,* which is located upstream of *lrgAB*. It has been shown that in *S. aureus* the *lytRS* operon is a positive regulator of *lrgAB* genes, since the *lrgAB* genes were not expressed in a *lytRS* mutant (Brunskill & Bayles, 1996).

Various *cid*, *lrg* and *lyt* operon homologues have been found in several gram-positive bacteria, including *Bacillus* sp., *Enterococcus faecalis* and *S. mutans*. The *S. mutans cid* operon comprises two genes, SMU.1701c (*cidA*) and SMU.1700 (*cidB*). The amino acid sequences of *lrgA*- and *lrgB*- encoded proteins are 38% and 49% identical to the *S. aureus* LrgA and LrgB proteins, respectively and those of *cidA* and *cidB* have 40% and 30% identity to their *S. aureus* counterparts. Like in *S. aureus*, the *lytSR* genes are located immediately upstream of *lrgAB*. The *S. mutans lytSR* gene products belong to the two-component signal transduction systems, and comprise a sensor histidine kinase located in the membrane (LytS) and a response regulator located in the cytoplasm (LytR).

Recently, it has become evident that programmed bacterial cell death is important for biofilm formation (Bayles, 2007). Following cell death, bacteria release DNA, which can have an important role in intercellular adhesion and biofilm stability by serving as a matrix that holds the biofilm together. A mutant of *S. mutans* deficient in binding and uptake of DNA showed reduced biofilm formation (Petersen *et al.*, 2005). Furthermore, biofilms could provide the selective pressure needed for programmed cell death by eliminating damaged bacteria from the population, thus enhancing nutrient availability for the remaining healthy individuals (Bayles, 2007). Bacterial autolysis also plays an important role in daughter cell separation and peptidoglycan remodelling; therefore its activity must be tightly controlled (Blackman *et al.*, 1998; Madiraju *et al.*, 1987).

In order to investigate the role of these genes in regulated cell death and biofilm formation of *S. mutans*, *lrgAB*, *cidAB*, *lytR* and *lytSR* mutant strains were generated by replacement mutagenesis and characterized.

3.2.2 Analysis of the expression of *lrgA*, *cidA* and *lytRS* genes

To investigate whether *lytSR* controls the expression of the *lrgA* gene in *S. mutans*, quantitive reverse transcriptase PCR was performed with RNA of the wild type strain (OMZ1001) and of the *lytR* and *lytSR* mutants grown planktonically in THYE medium. Samples were taken at the beginning of the exponential phase (OD_{600}=0.1), the mid-exponential phase (OD_{600}=0.4), the beginning of stationary phase (OD_{600}=0.8) and the stationary phase (OD_{600}=1.5). As shown in **Figure 18**, in the wild type strain,

lrgA was minimally expressed at the beginning of growth.

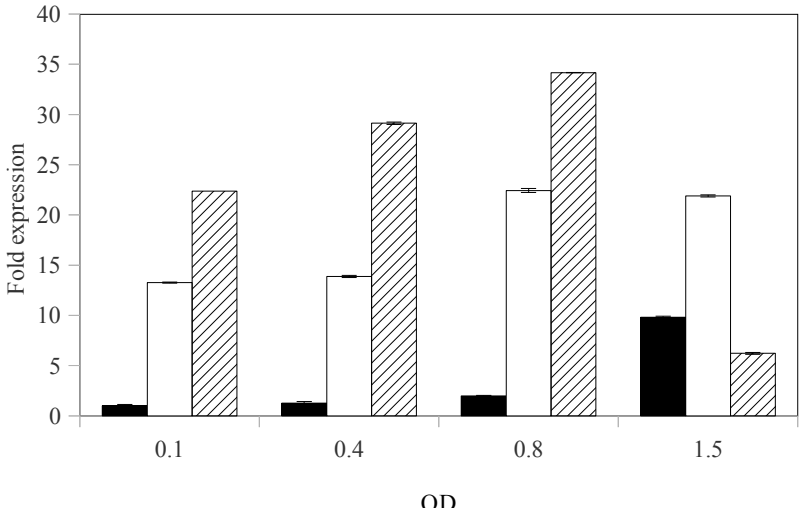

Figure18. Expression of *lrgA* during different phases of planktonic growth by the wild type (black bars), the *lytR* (white bars) and the *lytSR* mutant strains (striped bars).

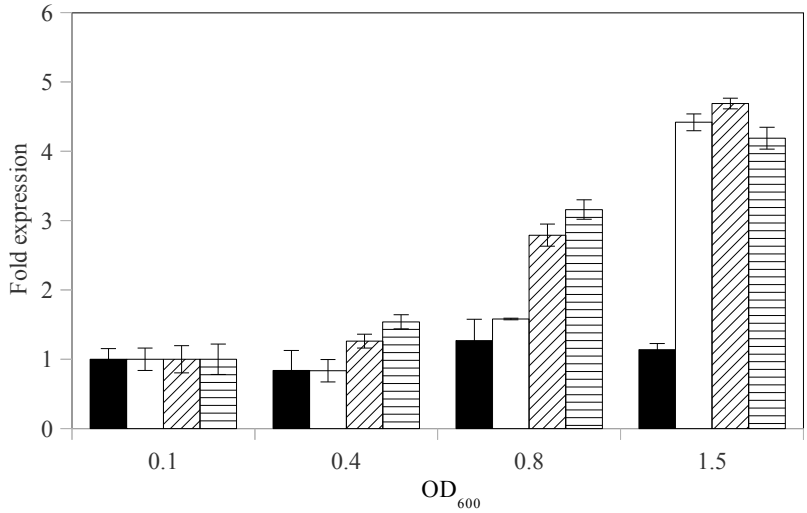

Figure 19. Expression of *cidA* (black bars), *lrgA* (white bars), l*ytR* (striped bars) and *lytS* (horizontal bars) genes in the wild type strain during different phases of planktonic growth.

A significant increase in expression was observed when bacteria reached the stationary phase. In contrast, in the *lytR* mutant, *lrgA* expression was de-repressed during the entire growth. This suggests that *lytR* is negative regulator of *lrgAB* expression. In the *lytSR* mutant, expression of lrgA was also de-repressed, except in the stationary phase. To investigate further the expression of *lytS, lytR* and *cidA* genes during growth, quantitative PCR was employed with the same samples from the wild type strain

and from the *lytSR* mutant. As shown in **Figure 19**, both *lytS* and *lytR* genes showed the same pattern of expression as *lrgA* in the wild type strain. Their expression steadily increased, being most highly expressed in the stationary phase. In contrast, *cidA* showed no difference in expression throughout the entire growth curve in the wild type strain.

3.2.3 Biofilm formation by mutants

To assess possible differences in the biofilm growth of the mutants, bacteria were grown for 24 hours in 24-well polystyrene plates in 3 different types of media supplemented with either glucose or sucrose. After 24 hours, supernatants were discarded; biofilms were washed and stained with crystal violet. For the wild type strain, only little biofilm was formed in THYE or in FUM medium containing glucose. More biofilm was formed in THYE medium or BM medium containing glucose, but the highest amount of biofilm was formed in medium containing sucrose. However, there were no differences between the wild type and the mutants while growing as biofilms (**Figure 20**).

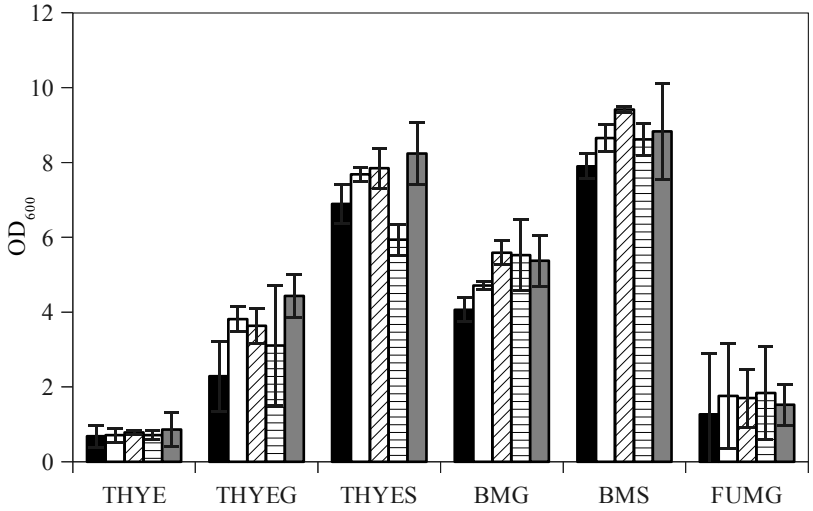

Figure 20. Biofilm formation of wild type and mutants. Wild type (black bars), *lrgAB* (white bars), *cidAB* (striped bars), *lytR* (horizontal striped bars) and *lytSR* mutants (grey bars) were grown in THYE, THYEG (THYE + 0.3% glucose), THYES (THYE + 0.5% sucrose), BMG (semi-defined biofilm medium containing 0.8% glucose), BMS (semi-defined biofilm medium containing 0.8% sucrose) and FUM (Fluid universal medium containing 0.3% glucose) and stained for biofilm formation by crystal violet.

3.2.4 Mutation of *lrgAB* affects stationary phase survival

Given that (i) the *lrgAB* and *lytSR* operons were most highly expressed in the stationary phase, (ii) the constitutive expression of *lrgA* in both *lytSR* and *lytR* mutants and (iii) the high expression of *lrgAB* in starving biofilms (microarrays 5 and 6), the question was addressed whether mutation of either the *lrgAB*, *cidAB* or *lytSR* genes will affect stationary phase survival in sugar-limited medium. Planktonic cultures were incubated at 37°C for 13 days. Aliquots of the cultures were taken at days 1, 2, 3, 4, 6, 9, 11 and 13, sonicated, diluted and plated onto THYE agar. The plates were incubated at 37°C for 48 hours. As shown in **Figure 21,** the wild type strain and the *cidAB* and *lytSR* mutant strains displayed similar levels of viability over the entire period. For all strains there was an initial drop in viability after 3 days that is after entry into the stationary phase. At day 3 the viable counts were between 10^8-10^9 CFU/ml, except for the *lytR* mutant, which contained more than 10^9 CFU/ml. Only the *lrgAB* mutant strain showed a significantly reduced survival after 4 days. At day 13, the viable counts were approximately 3 $\log_{10}$–units lower than those of the wild type strain. This could indicate that *lrgAB* genes are required for the long-term persistence of bacteria under sugar limitation. The *lytR* mutant exhibited a higher cell count in the first 4 days of growth, and than dropped to levels similar to the wild type.

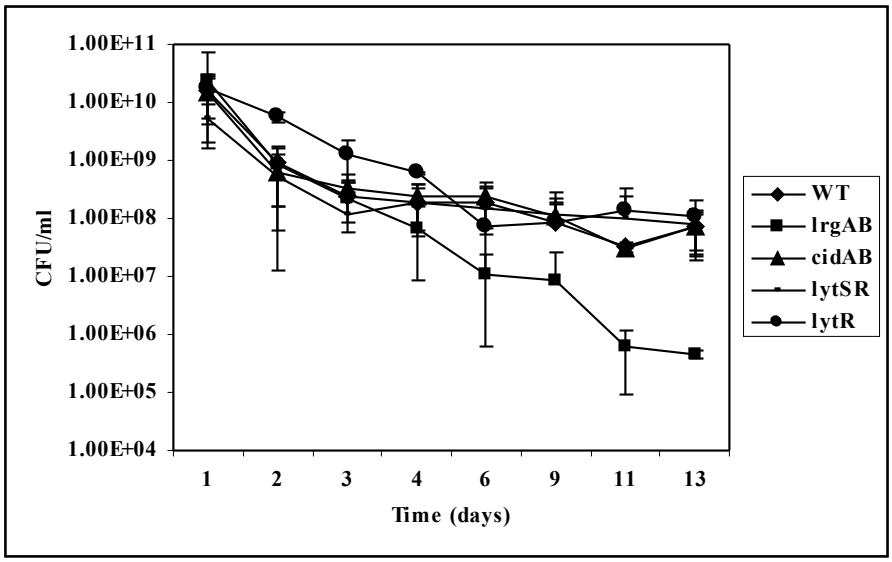

Figure 21. Long-term survival of wild type and mutant strains in planktonic cultures.

3.2.5 Expression of *lrgA*, *cidA* and *lytS* in the presence of acids

In part I of the results it was shown that expression of *lrgA* and *lrgB* expression was strongly

induced in stationary phase biofilms in medium supplemented with glucose compared to stationary phase biofilms in medium supplemented with sucrose (20-fold for *lrgA* and 6-fold for *lrgB*). In addition, *lrgA* and *lrgB* were also induced in starved biofilms. *S. mutans* produces high amounts of lactic acid as a result of carbohydrate metabolism. Since there was no significant difference in the final pH of biofilms grown in medium supplemented with glucose (4.4-4.5) compared with biofilms grown in medium supplemented with sucrose (4.3), it was of interest to test whether acids had an influence on the expression of *lrgA*, *cidA* and *lytS*. For this, a culture of the wild type strain grown in THYE was supplemented with either 30 mM of acetic acid, lactic acid, pyruvic acid or formic acid and incubated for one hour. The relative amounts of *lrgA*, *cidA* and *lytS* transcripts were determined by using qRT-PCR. Since addition of the acids resulted in a decrease of the pH of the medium to about 5, as control the wild type strain was also incubated in medium whose pH had been adjusted to 5.0 by addition of HCl.

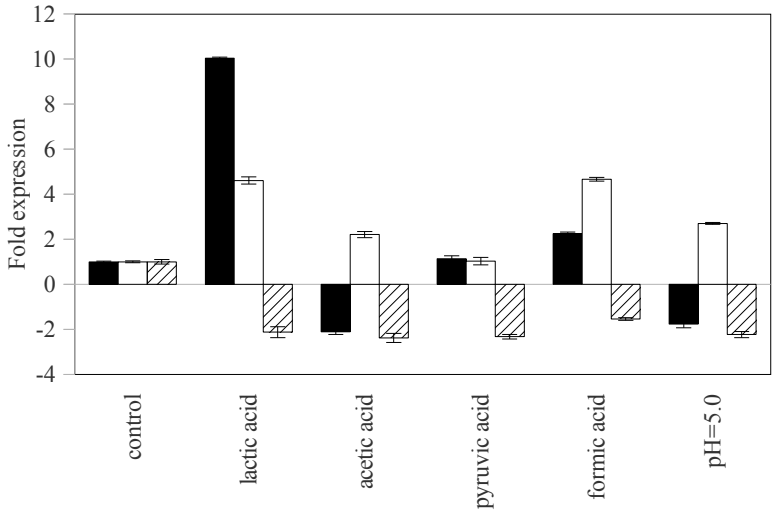

Figure 22. Expression of *lrgA* (black bars), *cidA* (white bars) and *lytS* (striped bars) in response to acids.

As illustrated in **Figure 22**, *lrgA* showed a 10-fold and 2.2-fold increase in expression in response to lactic acid and to formic acid respectively. In the presence of acetic acid the expression of *lrgA* was down-regulated 2.1-fold, which was similar to the down-regulation observed in the medium adjusted to a pH of 5.0. Pyruvic acid had no influence on the expression of *lrgA*. The expression of the *cidA* gene was increased between 2.2- and 4.6-fold for all conditions tested, except for the culture with pyruvic acid, where the expression of *cidA* had not changed. The expression of *lytS* was down-

regulated under all conditions, suggesting that low pH reduces the synthesis of LytS.

3.2.6. Expression of *lrgA*, *cidA* and *lytS* in response to CCCP

It has been shown that weak acids, such as lactic acid, can act as uncoupling agents (Baronofsky *et al.*, 1984; Herrero *et al.*, 1985). It was therefore investigated whether carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an artificial uncoupling agent which dissipates the proton-motive force across the membrane, influences the expression of *lrgA*, *cidA* and *lytS*. RNA was extracted from cultures which had been exposed to 100 µM of CCCP during 30 minutes and qRT-PCR was used to analyze the levels of *lrgA*, *cidA* and *lytS* transcripts (**Figure 23**). Expression of *lrgA* was increased by 6-fold in the wild type strain. However, in both the *lytR* and *lytSR* mutants *lrgA* expression was not changed after exposure to CCCP, suggesting that *lytSR* is required for the expression of *lrgA* in response to the CCCP. The expression of both *cidA* and *lytS* was slightly increased in all strains after CCCP exposure (**Figure 23**).

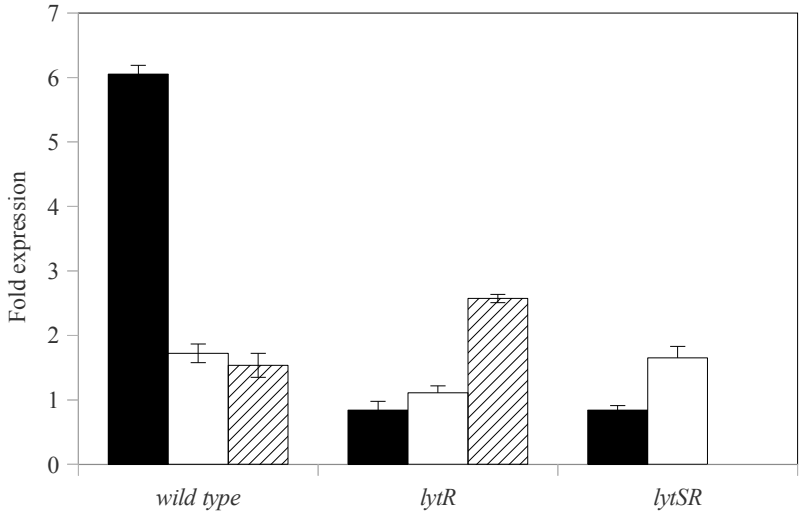

Figure 23. Expression of *lrgA* (black bars), *cidA* (white bars) and *lytS* (striped bars) in wild type and in *lytR* and *lytSR* mutants exposed to 100 µM of CCCP.

3.2.7 Chain length is increased in the *lytR* mutant

As the initial goal was to determine whether *lrgAB, cidAB* or *lytRS* operons are involved in autolysis or cell death in *S. mutans*, it was investigated whether the mutations had an influence on chain length. For each strain, the number of cells per chain was counted for more than 200 chains (**Figure**

24). The *lytR* mutant displayed a significantly longer chain size than the wild type strain and the other mutants. In the *lytR* mutant, 50% of chains contained 6 to 10 cells compared to 22% of the chains of the wild type strain. Moreover, 10% of the chains of the *lytR* mutant contained between 11-15 cells and 5% of the chains had up to 20 cells. In the wild type, the percentage of chains with 11-15 cells/chain and 16-20 cells/chain was below one (**Figure 24**). These data clearly suggest that the *lytR* mutant has a defect in daughter cell separation upon division.

3.2.8 Growth of wild type and *lytSR* mutant at pH=5.0

To further decipher the response of *lrg* and *lyt* genes to an acidic environment, the mutants were grown on solid medium at pH=7.0 and pH =5.0. Dilutions were made and 20 µl of each dilution was spotted onto THYE agar plates, which were incubated for 24 hours. The colonies of the wild type were noticeably smaller at pH 5.0 (**Figure 25**). This was also the case for the *cidAB*, *lrgAB* and *lytR* mutants (data not shown). However, the colonies of the *lytSR* mutant were clearly larger than the wild type strain at pH 5.0, whereas the colony size was similar at pH 7.0 (**Figure 25**).

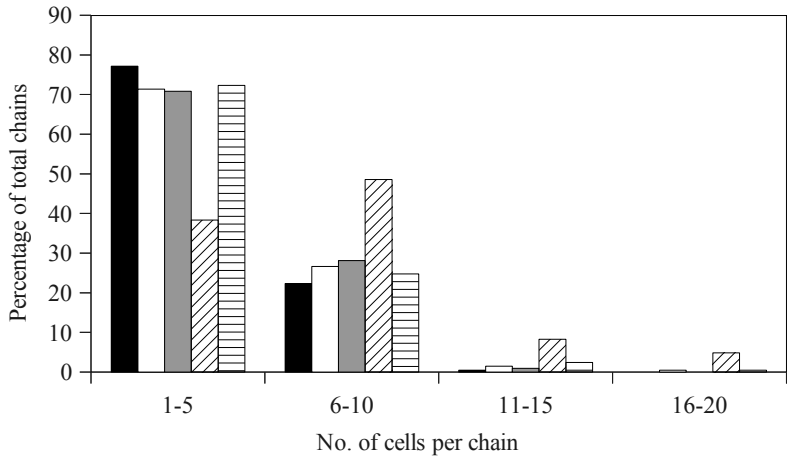

Figure 24. Quantification of chain length of wild type (black bars) and *lrgAB* (white bars), *cidAB* (grey bars), *lytR* (striped bars) and *lytSR* mutants (horizontal bars) based on phase contrast microscopy.

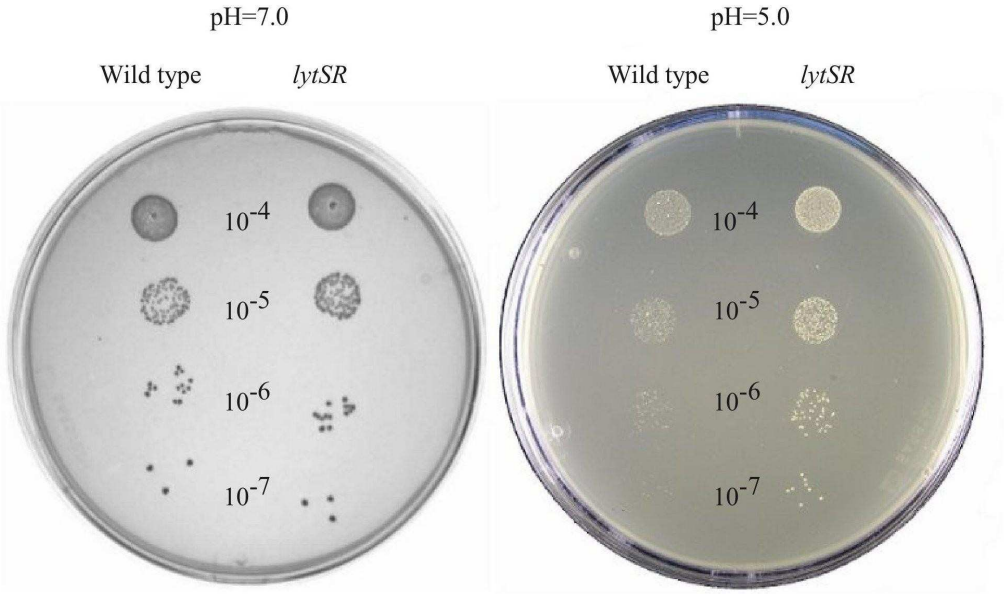

Figure 25. Growth of wild type and *lytSR* mutant at pH=7.0 and pH=5.0. Different dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) of cultures of the wild type strain and the *lytSR* mutant (*lytSR*) were spotted on THYE agar plates at pH=7.0 or pH=5.0.

3.2.9 Discussion

The capacity of *S. mutans* to persist in biofilms is a key factor for survival in the oral cavity. Using transcriptomic analysis to screen for *S. mutans* genes that might have an important role in biofilm formation and maintenance, the *lrgAB* genes were identified as being highly expressed in stationary phase biofilms. Most interestingly, these genes showed a very high expression in biofilms that were starved. In contrast, in biofilms grown in sugar-containing media, the expression of *lrgAB* genes was drastically reduced. To address the question why *lrgAB* genes are highly expressed under starvation conditions, a stationary phase survival experiment was performed. Among all mutant strains tested, only the *lrgAB* mutant showed a significant decrease in survival. This could indicate that the *lrgAB* genes are required for survival in the stationary growth phase under carbohydrate-limiting conditions. A *Bacillus anthracis lrgAB* mutant also showed decreased survival in stationary phase (Chandramohan *et al.*, 2009). Like in *B. anthracis*, the *lrgAB* mutation in *S. mutans* had no effect on murein hydrolase activity or on autolysis (data not shown). In *S. aureus* though, an *lrgAB* mutant exhibits increased murein hydrolase activity and increased autolysis (Groicher *et al.*, 2000).The mechanism by which the proteins encoded by *lrgAB* mediate stationary phase survival in *S. mutans* and

in *B. anthracis* is probably different from that in *S. aureus*.

In the wild type strain, the expression of *lrgA* and *lrgB* is up-regulated in the stationary phase of growth. In *S. aureus* it has been shown previously that expression of *lrgAB* is highest when cells are approaching stationary phase (Groicher *et al.,* 2000). Expression of *lrgAB* in *S. aureus* is positively regulated by the LytSR two-component signal transduction system (TCS). Here, we have shown that the expression of *lrgAB* in *S. mutans* is negatively regulated by LytSR since in both the *lytR* and *lytSR* mutants *lrgA* expression was de-repressed.

Consistent with LytSR negatively regulating *lrgAB*, inactivation of *lytSR* or *lytR* had no effect on stationary phase survival. But mutants in *lytR* and *lytSR* showed some other interesting phenotypes. The chains of the *lytR* mutant were longer than those of the wild type strain. This suggests that the LytSR TCS may be involved in cell-cell separation. However, the *lytSR* mutant had a chain length like the wild type strain, which seems to contradict this hypothesis. On the other hand, the *lytSR* mutant, but not the *lytR* mutant, showed an increase in colony size on solid medium at pH 5.0 compared with the wild type strain. (Levesque *et al.*, 2007; Biswas *et al.*, 2008) demonstrated that inactivation of *lytS* had no effect on the acid tolerance response of *S. mutans*, but (Kawada-Matsuo *et al.*, 2009) found that either mutation of *lytS*, *lytR* or both *lytS* and *lytR* led to a decrease in growth rate at pH 5.5. These apparently contradictory results, including those presented here, require further investigations.

In *S. aureus* the *lrgAB*-encoded proteins are called “antiholins” and are predicted to have a protective function against cell death or lysis by counteracting the function of “holins”. The holins, encoded by *cidAB*, are thought to promote release of murein hydrolase. In *S. aureus*, expression of *cidAB* genes is highest in the beginning of the exponential growth phase and regulated by the LysR-type transcriptional regulator CidR, which is encoded upstream of *cidAB* (Rice *et al.*, 2003; Yang *et al.*, 2005). In contrast, in *S. mutans*, expression of *cidA* was found to be independent of the growth phase. The *cidR* gene appears to be absent, which suggests a different kind of regulation. In addition, the *cidAB* mutant of *S. mutans* had a phenotype which was indistinguisable from the wild type. The function of *cidAB* is therefore at present not clear.

In bacteria, many external signals are transmitted by TCS. Upon sensing the signal, a histidine kinase activates through phosphorylation the response regulator, which modulates the transcription of target genes. In *S. mutans*, 14 TCS have been identified, but only a few have been characterized (Senadheera *et al.*, 2009; Suntharalingam *et al.*, 2009). Even less is known about the signals to which the TCS respond. It was attempted to gain more information on the environmental signals which can

trigger the activation of the *lytRS* two-component signal transduction system and its action on the *lrgAB* genes. In *S. mutans,* glucose metabolism generates a mixture of organic acids such as lactic acid, acetic acid and formic acid. Lactic acid was the most potent acid that induced *lrgAB*, followed by formic acid. Acetic acid was not an inducer of *lrgAB* in the conditions tested. Dashper and Reynolds showed that the addition of 50 mM or 100 mM lactate and formate to a streptococcal culture at pH= 6.3 and pH= 6.0 significantly reduced growth, whereas the addition of the same amount of acetate had no effect (Dashper & Reynolds, 2000).

Although lactic acid induces *lrgAB* and *cidAB* expression most likely by dissipation of the proton gradient across the membrane (Dashper & Reynolds, 2000), it has also been shown in other bacteria that organic acids can act as uncoupling agents affecting both components of proton motive force (PMF): proton gradient and membrane potential (Baronofsky *et al.*, 1984).

The exposure of *S. mutans* to CCCP, an ionophore that affects both components of PMF resulted in high expression of *lrgAB* but not *cidAB*. This could indicate that *lrgAB* respond to changes in PMF, whereas *cidAB* respond only to changes in the ΔpH. It could be shown that the *lrgAB* response to CCCP is dependent on LytSR, since in *lytSR* and *lytR* mutants the expression of *lrgAB* genes did not change compared with the wild type. It can thus be hypothezised that the kinase *lytS* responds to changes in the membrane status of *S. mutans*.

Part III. *Streptococcus mutans* PknB is involved in regulation of genetic competence, bacteriocins production and cell-wall metabolism.

3.3.1 General aspects

All living organisms have constantly to adapt to changes in the environment. In bacteria, signals from the outside world are frequently detected and transmitted by two-component systems (TCS), which consist of a membrane-located sensor histidine kinase and response regulator which is localized in the cytoplasm. In response to an environmental signal, the former protein is autophosphorylated. The phosphate group is then transferred to an aspartate residue of the response regulator which, in its active phosphorylated form, regulates the transcription of target genes. *S. mutans* contains 14 TCS (Biswas *et al.*, 2008; Levesque *et al.*, 2007), some of which have been studied in detail. The ComDE TCS is essential for expression of bacteriocins (van der Ploeg, 2005) and involved in development of genetic competence (Li *et al.*, 2002b). The VicKR TCS is required for response to oxidative stress (Deng *et al.*, 2007; Senadheera *et al.*, 2007c).

Another type of signal transduction system comprises serine/threonine protein kinases (STPKs), which were first discovered in eukaryotes, but later found to be widespread in bacteria as well. Most of the bacterial STPKs are predicted to consist of an N-terminal kinase domain in the cytoplasm, a central membrane-located domain and a C-terminal sensory domain located extracellularly. Like histidine kinases, STPKs are autophosphorylated but in this case on serine or threonine residues. In contrast to two-component response regulators, STPKs exert their effect by phosphorylation of other proteins. Prokaryotic STPKs have been shown to regulate various cellular functions including virulence in streptococci (Echenique *et al.*, 2004; Jin & Pancholi, 2006; Rajagopal *et al.*, 2003). Dephosphorylation of STPKs is thought to be carried out by their cognate serine-threonine protein phosphatases (STPPs), which was confirmed *in vitro* (Boitel *et al.*, 2003; Jin & Pancholi, 2006; Nováková *et al.*, 2005; Rajagopal *et al.*, 2003). Thus, STPPs might reverse the effect of STPKs.

S. mutans contains one STPK and one STPP, encoded by *pknB* and *pppL* respectively (Hussain *et al.*, 2006). Mutation of *pknB*, which is located immediately downstream of *pppL*, results in a pleiotropic phenotype: it causes defects in the development of genetic competence, in the ability to form biofilms and in tolerance to low pH (Hussain *et al.*, 2006).

In the present study, we have carried out further phenotypic investigations of *pknB*, *pppL* and

pknB pppL double mutants. Microarray analysis was used to analyze the transcriptome of a *pknB* mutant. It is shown that the *pknB* regulon exhibits overlap with the *vicRK* regulon and *comDE* regulons, suggesting cross-talk between PknB and these TCS.

3.3.2 Aberrant cell division in the mutants

Transmission electron microscopy showed that cells of the *pknB* mutant (**Figure 26**) had an abnormal shape. They were much larger, more rounded or oval compared with the wild type strain (**Figure 26, B** and **F**). As opposed to the wild type strain, a significant fraction (about 10%) of the cells of the *pknB* mutant appeared to have lysed. Cells of the PPPL mutant had the same size as the wild type, but were also more rounded and showed irregular cell division. In many PPPL cells the division plane was in an angle or perpendicular to the division plane from a previous division (**Figure 26**, **C** and **G**). Strain PKPL (**Figure 26, D** and **H**) appeared similar as strain PKNB, except for the apparent absence of lysed cells. These results suggest defects in cell shape and in cell division in both the *pknB* and the *pppL* mutant.

3.3.3 Transcriptome analysis of a *pknB* mutant (Microarray 7)

To identify genes whose expression is under direct or indirect control of PknB, the transcriptomes of strains UA159 and PKNB grown to the exponential growth phase (OD_{600}= 0.3) were compared. Data analysis revealed 26 up-regulated genes and 41 down-regulated genes in the *pknB* mutant (**Table 4**).

Among the up-regulated genes in the *pknB* mutant were several which are likely to be involved in synthesis, degradation or remodeling of the cell-wall. Expression of SMU.609, a putative murein hydrolase (Catt & Gregory, 2005), was up-regulated more than three-fold in the *pknB* mutant. SMU.20 and SMU.21, which encode the putative cell-shape determining proteins MreC and MreD respectively, were also up-regulated. Depletion of MreC or MreD in *Bacillus subtilis* resulted in altered cell shape (Mark & Errington, 2005), whereas a *mreD* mutant of *S. thermophilus* formed very long chains and was sensitive to oxidative stress (Thibessard *et al.*, 2004). One of the most strongly up-regulated genes, SMU.984, shows similarity to several autolysins and *N*-acetylmuramoyl-L-alanine amidases. Although its function is unknown, SMU.984 contains a CHAP domain (Bateman & Rawlings, 2003), suggesting that it might have cell-wall degrading activity. However, a signal sequence for translocation across the membrane could not be detected (data not shown). The genomic location of SMU.984 is somewhat

peculiar in the context of its possible function, since it is located within a cluster of genes required for β-glucoside metabolism (Cote *et al.*, 2000). SMU.984 is preceded by a canonical *E. coli* σ^{70}-dependent promoter and followed by a transcriptional terminator. Nevertheless, the gene downstream of SMU.984, *bglA*, was also up-regulated in the *pknB* mutant.

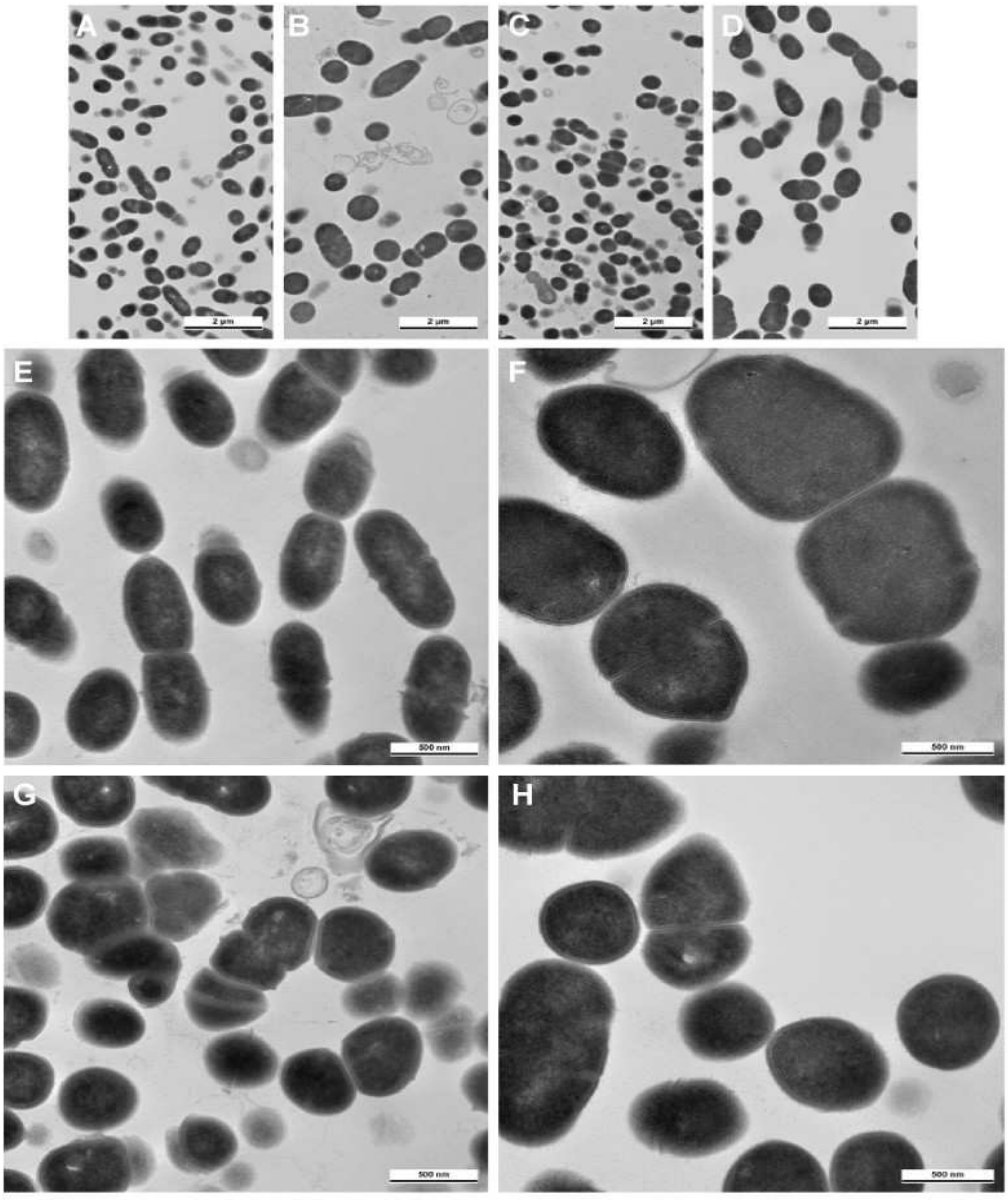

Figure 26. Transmission electron microscopy of *S. mutans* wild type (A, E) and the mutants PKNB (B, F), PPPL (C, G) and PKPL (D, H). Magnification of A, B, C and D is 2500x Magnification of E, F, G and H is 12000x.

SMU.503c, encoding a probable lipoprotein of unknown function, was up-regulated nearly five-fold in the mutant. The two ORFs adjacent to SMU.503c are transcribed in the opposite direction, which implies that SMU.503 is in a single gene operon. Other up-regulated genes included SMU.1067c, a component of an ABC transporter with unknown substrate, and SMU.334 and SMU.335, involved in biosynthesis of arginine. SMU.1895c and SMU.1896c, probably encode bacteriocins (van der Ploeg, 2005), were strongly down-regulated in the *pknB* mutant. The expression of several other bacteriocins or bacteriocin-related genes was down-regulated as well (SMU.1906, SMU.1908, SMU.1912 and SMU.1914). A cluster of genes encoding small proteins (SMU.277, SMU.278, SMU.279, SMU.281, SMU.283 and SMU.285) were all down-regulated in the *pknB* mutant. The function of these genes is unknown but of possible significance is that they are located immediately upstream of the *comAB* genes, which are required for export of CSP (van der Ploeg, 2005). Interestingly, using BAGEL (de Jong *et al.*, 2006) all six genes were predicted to encode bacteriocins.

SMU.2146c, whose amino acid sequence shows similarity to transglycosylases involved in cell-wall remodeling, was down-regulated by about five-fold in the *pknB* mutant. Six genes from the *comY* operon (Merritt *et al.*, 2005b) were down-regulated in the *pknB* mutant by 2- to 5-fold. These genes are directly involved in uptake of DNA during competence (Merritt *et al.*, 2005b). Two other genes, SMU.625 and SMU.499, putatively encoding functions in competence development, were down-regulated as well.

Other down-regulated genes included SMU.1062 (*opuAA*), SMU.1063 (*opuAB*) and SMU.2116 (*opuCA*). These genes are thought to encode ATP-binding cassette (ABC) transporters for uptake of compatible solutes and have been shown to be induced by osmotic stress (Abranches *et al.*, 2006). In addition, two PTS transport systems, one for fructose/mannose (SMU.1956, SMU.1957, SMU.1958, SMU.1961 and SMU.1962) and one for trehalose (SMU.2037 and SMU.2038), were down-regulated in the *pknB* mutant.

Reverse-transcriptase real-time PCR of 12 genes was performed to validate the results from microarray analysis (**Figure 27**). A good correlation between both methods was observed (r^2=0.97) (**Figure 28**).

3.3.4 Bacteriocin production in mutants

Production of bacteriocins was investigated *in vivo* by using deferred antagonism assays with *Enterococcus faecalis* OMZ 940 as indicator strain. The *pknB* and the double *pknB pppL* mutant, but

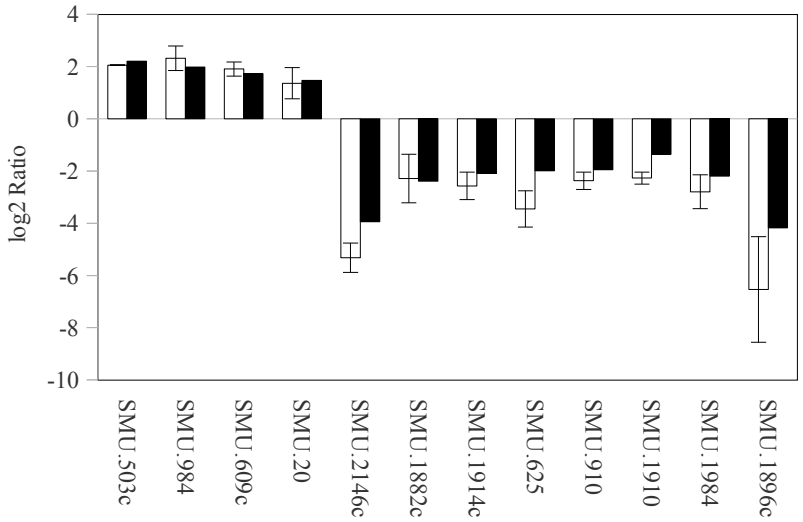

Figure 27. Validation of microarray results with quantitative real time PCR. Data are generated from two independent RNAs, each in triplicate. White bars: qRT-PCR ratios, black bars: microarray ratios.

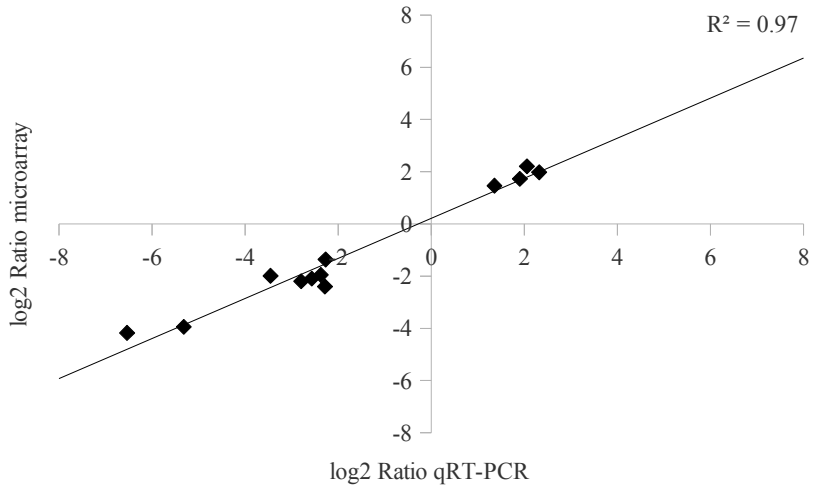

Figure 28. Correlation of gene expression between microarray and qRT-PCR. Each point represents one gene. Fold changes in gene expression after comparison of wild type and pknB mutant are plotted. R^2 indicates linear regression.

not the *pppL* mutant, showed a strong reduction in inhibition (**Figure 29**). The *ciaRH* two-component signal transduction system is known to regulate competence development and bacteriocin production in *S. mutans* (Ahn *et al.*, 2006; Qi *et al.*, 2004). A mutant in which *pknB* and both *ciaR* and *ciaH* had been inactivated, inhibited growth of *E. faecalis* OMZ 940 like the wild type strain. Thus, deletion of *ciaRH* appears to counteract the *pknB* defect. Whereas the wild type strain is immune against its own bacteriocins, the *pknB* mutant was sensitive to the bacteriocins produced by the wild type strain.

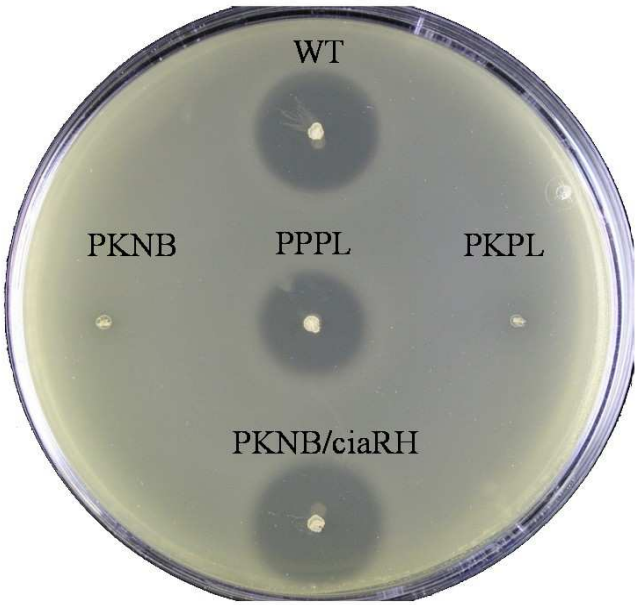

Figure 29. Inhibition of *E. faecalis* OMZ940 by *S. mutans.*

3.3.5 Paraquat exposure

It was shown previously that the *pknB* mutant is sensitive to low pH, having a reduced growth when grown on THYE agar plates at pH=5.0 (Hussain *et al.*, 2006). To further investigate if strain PKNB is also affected by reactive oxygen species, it was grown in the presence of paraquat. Addition of 25 mM paraquat to the growth medium (THYE) resulted in an increase of the lag phase of the wild type strain of about 7 hours, whereas the *pknB* mutant did not grow at all (**Figure 30**).

3.3.6 Expression of PknB-regulated genes in TCS mutants

In order to investigate whether other two-component signal transduction systems (TCS) were involved in regulation of genes which were differentially expressed in the *pknB* mutant, real-time RT-

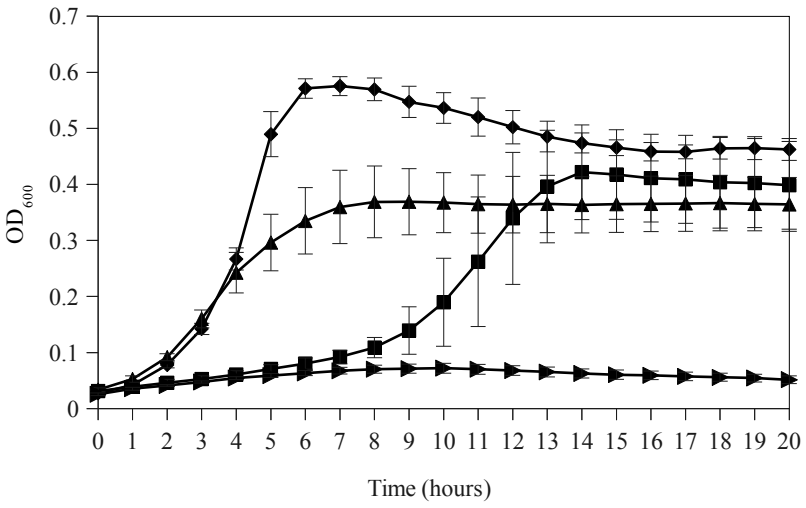

Figure 30. Growth of wild type and *pknB* mutant in the presence of 25 mM paraquat. Diamonds: wild type without paraquat; Squares: wild type with 25 mM paraquat; Triangles: *pknB* mutant without paraquat; Circles: pknB mutant with 25 mM paraquat.

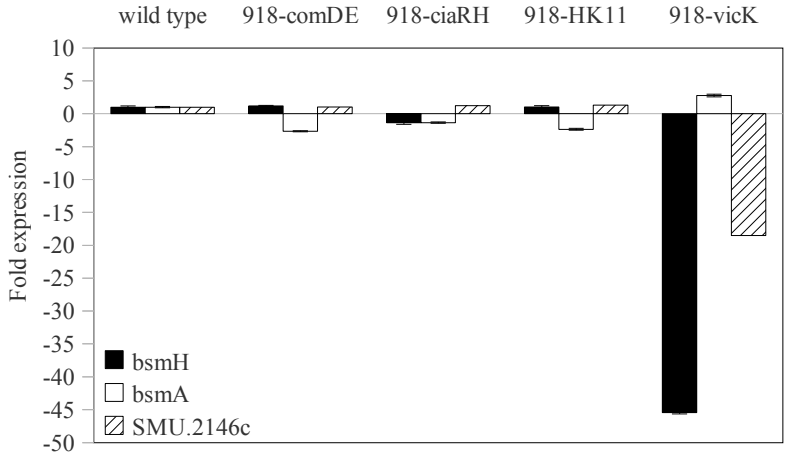

Figure 31. Expression of *bsmH*, *bsmA* and SMU.2146c in *comDE, ciaRH, hk11*, and *vicK* mutants.

PCR was employed to compare the expression of *bsmA*, *bsmHI* and SMU.2146c in the wild type strain and in mutants in TCS. RNA was extracted from cultures of *S. mutans* UA159 and derivatives thereof with mutations in *comDE*, HK11 (SMU.486), *vicK* and *ciaRH*. The expression of SMU.2146c and *bsmHI* was strongly down-regulated in the *vicK* mutant, but remained almost unchanged in the other mutants (**Figure 31**). On the other hand, the expression of *bsmA* was up-regulated in the *vicK* mutant,

but down-regulated in the *comDE* mutant. The down-regulation of *bsmA* in the *comDE* mutant corroborates previous results (van der Ploeg, 2005).

These results suggest that *vicK* exerts positive regulation on *bsmHI* and SMU.2146c. The cognate response regulator of VicK, VicR, recognizes a consensus binding site (Dubrac *et al.*, 2008). Inspection of the upstream sequences revealed the presence of a putative VicR binding site (tgttattttcttgcaattatttgactt) upstream of *bsmH* but not of SMU.2146c.

3.3.7 Expression of PknB-regulated genes in a *pppL* mutant

We hypothesized that the role of the putative PppL phosphatase is to counter the action of the PknB kinase. To validate this hypothesis, reverse transcriptase real-time PCR was used to analyse the expression levels of the PknB-regulated genes *bsmA*, *bsmH*, SMU.2146c, *comYC* and *mreC* in *pppL* mutant strain PPPL (**Figure 32**). Indeed, genes that were down-regulated in the *pknB* mutant were up-regulated in the *pppL* mutant. However, the *mreD* gene, which was up-regulated in the *pknB* mutant, was only slightly up-regulated in the *pppL* strain.

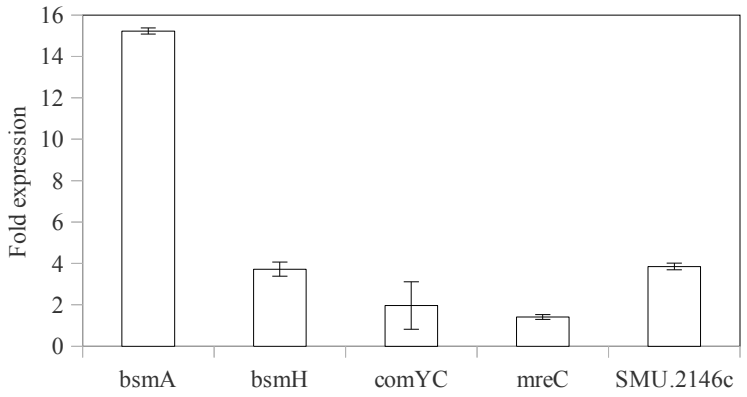

Figure 32. q RT-PCR analysis of expression of selected genes in the *pppL* mutant.

3.3.8 Discussion

The results from this and a previous study (Hussain *et al.*, 2006) have shown that the *pknB* mutant of *S. mutans* is deficient in several virulence factors: acid tolerance, oxidative stress and production of bacteriocins. Electron microscopic analysis revealed aberrant cell sizes and shapes of the *pknB* mutant cells and of the double mutant, which may render them more susceptible to different stress conditions, such as low pH, oxidative and osmotic stress. Cells of the *pppL* mutant had a normal

size, but appeared affected in cell division. Analysis of the growth of the mutant strains in broth showed that they are retarded to varying degrees (data not shown), indicating the importance of the system for normal growth.

The mutants showed defects in their ability to grow at low pH, an environmental stress frequently encountered by *S. mutans* within the oral cavity (Elaine Allan, personal communication). In addition, the *pknB* mutant was unable to grow under conditions of oxidative stress. The *S. pneumoniae stkP* mutant also shows a reduced tolerance of acidic pH and also other environmental stresses including oxidative stress, high temperature and high osmolarity (Sasková *et al.*, 2007), suggesting that these signal transduction systems are concerned with transmitting information about environmental signals into the cell.

As *pppL* is located upstream of *pknB* and the two are co-transcribed, an insertion in *pppL* could affect the expression of *pknB*. In this work, we showed that the phosphatase mutant and the double mutant have different phenotypes indicating that the insertion in *pppL* does not abolish expression of *pknB*. The presence of a transcriptional terminator downstream of *pknB* (Hussain *et al.*, 2006), suggests that polar effects on genes further downstream are unlikely.

The phenotype of the *pknB pppL* double mutant very was similar to that of the single *pknB* mutant. It is interesting that the *S. mutans* kinase-phosphatase double mutant grew better than the phosphatase mutant; this suggests that inactivation of the kinase relieves some of the highly deleterious effects caused by mutation of *pppL*. These results are consistent with a model where the phosphatase PppL is required to return PknB to the unphosphorylated state and to keep a balance between phosphorylation and dephosphorylation. Excess of phosphorylated PknB, caused by the absence of phosphatase activity in the *pppL* mutant, is apparently harmful to the cell. In *S. pneumoniae, Streptococcus agalactiae* and *Streptococcus pyogenes,* mutation of the orthologous STPP gene is apparently lethal (P. Branny, personal communication, (Jin & Pancholi, 2006; Li *et al.*, 2002b). In future, it will be important to investigate whether the activity of STPPs is regulated and, if so, by which signals.

It has been shown before that the ComDE TCS is essential for the expression of a number of bacteriocin-encoding genes (Kreth *et al.*, 2007; van der Ploeg, 2005). ComDE are also involved in development of competence, but the mechanism by which competence is induced has not yet been elucidated. Transcriptome analysis showed that genes required for genetic competence and genes encoding bacteriocins were down-regulated in the *pknB* mutant. We also observed that the expression

of *bsmH* (SMU.1896c) and SMU.2146c was down-regulated in both *pknB* and *vicK* mutants. These results suggest that the PknB/PppL regulatory circuit interacts with the ComDE and VicKR TCS. Two different models can be envisaged for this interaction. Phosphorylation by PknB of as yet unknown target proteins might cause changes in the signals perceived by ComDE or VicKR. Alternatively, PknB might act directly by phosphorylation of the sensor kinase or the response regulator of the TCS. Phosphoproteomic studies have been used to determine the substrates for STPKs (Nováková *et al.*, 2005; Soufi *et al.*, 2008; Sztajer *et al.*, 2008), but no indication was found for phosphorylation of a TCS. However, it has recently been shown that *S. agalactiae* STPK Stk1 can phosphorylate the CovR response regulator *in vitro* at a threonine residue located in close vicinity to the aspartate residue phosporylated by the CovS histidine kinase (Rajagopal *et al.*, 2006). Phosphorylation by Stk1 prevented CovR from binding to the promoter (Jung Lin *et al.*, 2009).

Previous studies have shown that the VicRK TCS is involved in response to oxidative stress (Biswas *et al.*, 2008; Deng *et al.*, 2007; Senadheera *et al.*, 2005). VicRK was recently shown to be also involved in acid survival, since a *vicK* mutant produced less lactic acid and was more resistant to acid stress than the wild type strain (Senadheera *et al.*, 2009). Interestingly, transcriptome analysis revealed that SMU.1895c, SMU.1896c and SMU.2146c were among the most highly down-regulated genes in the *vicK* mutant grown at pH 5.5 (Senadheera *et al.*, 2009). Our RT-PCR data, although collected at neutral pH, confirm these results. Differential regulation of several other common genes in *vicK* and *pknB* mutants was observed (not shown), which suggests that the *vicKR* regulon overlaps at least in part with the *pknB* regulon. It is thus not surprising that there are similarities between the phenotypes of *pknB* and *vicK* mutants.

In *S. pneumoniae* overlap of genes regulated by VicRK and PknB has been observed as well (Sasková *et al.*, 2007). Notably, PknB and VicRK exert positive regulation on PcsB, an essential murein hydrolase involved in cell-wall synthesis (Ng *et al.*, 2004). For several streptococcal species, including *S. mutans* and *S. pneumoniae*, it has turned out impossible to isolate *vicR* mutants. It has therefore been proposed that VicR is essential for normal growth (Bhagwat *et al.*, 2001; Senadheera *et al.*, 2005; Wagner *et al.*, 2002). In *S. pneumoniae*, the requirement for VicR could be traced back to its function as transcriptional activator of *pcsB*, since *vicR* mutants which constitutively expressed *pcsB* were found to be viable (Ng *et al.*, 2003). In both *S. mutans* and *S. pneumoniae*, *vicK* mutants are viable, which suggests that phosphorylation of VicR is not solely dependent on the presence of VicK, but that VicR may be phosphorylated by other histidine kinases or by other phosphate donors such as

PknB. In agreement with the latter hypothesis, we were unable to obtain a *vicK pknB* double mutant of *S. mutans* (data not shown).

The defects of *pknB* and *pppL* mutants in cell shape and size and the differential expression of genes possibly involved in cell-wall biosynthesis allude to an important role of the system in sustaining cell-wall integrity. Most STPKs, including *S. mutans* PknB, contain a so-called PASTA domain, which is thought to bind unlinked peptidoglycan (Yeats *et al.*, 2002) and as a result could activate expression of cell-wall biosynthesis proteins (Yeats *et al.*, 2002). This hypothesis was recently confirmed for an STPK involved in exit for spore dormancy in *Bacillus subtilis* (Shah *et al.*, 2008). It will be of interest to determine whether the same molecule also functions as signal for PknB in *S. mutans*.

References

Aakra, A., Vebo, H., Snipen, L., Hirt, H., Aastveit, A., Kapur, V., Dunny, G., Murray, B. & Nes, I. F. (2005). Transcriptional Response of *Enterococcus faecalis* V583 to erythromycin. *Antimicrob Agents Chemother* **49**, 2246-2259.

Abranches, J., Chen, Y.-Y. M. & Burne, R. A. (2003). Characterization of *Streptococcus mutans* strains deficient in $EIIAB^{Man}$ of the sugar phosphotransferase system. *Appl Environ Microbiol* **69**, 4760–4769.

Abranches, J., Lemos, J. A. & Burne, R. A. (2006). Osmotic stress responses of *Streptococcus mutans* UA159. *FEMS Microbiol Lett* **255**, 240-246.

Ahn, S.-J., Wen, Z. T. & Burne, R. A. (2006). Multilevel control of competence development and stress tolerance in *Streptococcus mutans* UA159. *Infect Immun* **74**, 1631-1642.

Ahn, S.-J., Wen, Z. T. & Burne, R. A. (2007). Effects of oxygen on virulence traits of *Streptococcus mutans*. *J Bacteriol* **189**, 8519-8527.

Ajdić, D., McShan, W. M., McLaughlin, R. E., Savic, G., Chang, J., Carson, M.B., Primeaux, C., Tian, R., Kenton, S., Jia, H., Lin, S., Qian, Y., Li, S., Zhu, H., Najar, F., Lai, H., White, J., Roe, B.A. & Ferretti J.J. (2002). Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc Natl Acad Sci USA* **99**, 14434-14439.

Ajdić, D. & Pham, V. T. T. (2007). Global transcriptional analysis of *Streptococcus mutans* sugar transporters using microarrays. *J Bacteriol* **189**, 5049-5059.

An, D. & Parsek, M. R. (2007). The promise and peril of transcriptional profiling in biofilm communities. *Curr Opin Microbiol* **10**, 292-296.

Aoki, H., Shiroza, T., Hayakawa, M., Sato, S. & Kuramitsu, H. K. (1986). Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. *Infect Immun* **53**, 587-594.

Arirachakaran, P., Benjavongkulchai, E., Luengpailin, S., Ajdic, D. & Banas, J. A. (2007). Manganese affects *Streptococcus mutans v*irulence gene expression. *Caries Res* **41**, 503-511.

Barnes, M., Freudenberg, J., Thompson, S., Aronow, B. & Pavlidis, P. (2005). Experimental comparison and cross-validation of the Affymetrix and Illumina gene expression analysis platforms. Nucleic Acids Res **33**, 5914-5923.

Baronofsky, J. J., Schreurs, W. J. A. & Kashket, E. R. (1984). Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum*. *Appl Environ Microbiol* **48**, 1134-1139.

Bateman, A. & Rawlings, N. D. (2003). The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. *Trends Biochem Sci* **28**, 234-237.

Bayles, K. W. (2007). The biological role of death and lysis in biofilm development. *Nat Rev Microbiol* **5**, 721-726.

Bender, G. R., Thibodeau, E. A. & Marquis, R. E. (1985). Reduction of acidurance of streptococcal growth and glycolysis by fluoride and gramicidin. *J Dent Res* **64**, 90-95.

Bhagwat, S. P., Nary, J. & Burne, R. A. (2001). Effects of mutating putative two-component systems on biofilm formation by *Streptococcus mutans* UA159. *FEMS Microbiol Lett* **205**, 225-230.

Biswas, I., Drake, L., Erkina, D. & Biswas, S. (2008). Involvement of sensor kinases in the stress tolerance response of *Streptococcus mutans*. *J Bacteriol* **190**, 68-77.

Blackman, S. A., Smith, T. J. & Foster, S. J. (1998). The role of autolysins during vegetative growth of *Bacillus subtilis* 168. *Microbiol* **144**, 73-82.

Blencowe, B. J., Ahmad, S. & Lee, L. J. (2009). Current-generation high-throughput sequencing:

deepening insights into mammalian transcriptomes. *Genes Dev* **23**, 1379-1386.
Boitel, B., Ortiz-Lombardía, M., Durán, R., Pompeo, F., Cole, S. T., Cerveñansky, C. & Alzari, P. M. (2003). PknB kinase activity is regulated by phosphorylation in two Thr residues and dephosphorylation by PstP, the cognate phospho-Ser/Thr phosphatase, in *Mycobacterium tuberculosis*. *Mol Microbiol* **49**, 1493-1508.
Boyd, D. A., Cvitkovitch, D. G. & Hamilton, I. R. (1994). Sequence and expression of the genes for HPr (*ptsH*) and enzyme I (*ptsI*) of the phosphoenolpyruvate-dependent phosphotransferase transport system from *Streptococcus mutans*. *Infect Immun* **62**, 1156-1165.
Brady, L. J., Piacentini, D. A., Crowley, P. J., Oyston, P. C. & Bleiweis, A. S. (1992). Differentiation of salivary agglutinin-mediated adherence and aggregation of mutans streptococci by use of monoclonal antibodies against the major surface adhesin P1. *Infect Immun* **60**, 1008-1017.
Brazma, A. (2009). Minimum information about a microarray experiment (MIAME) – successes, failures, challenges. *TheScientificWorldJOURNAL* **9**, 420-423.
Brown, A. T. & Wittenberger, C. L. (1973). Mannitol and sorbitol catabolism in *Streptococcus mutans*. *Arch Oral Biol* **18,** 117-126.
Brunskill, E. W. & Bayles, K. W. (1996). Identification of LytSR-regulated genes from *Staphylococcus aureus*. *J Bacteriol* **178**, 5810-5812.
Burne, R. A., Chen, Y. Y. M., Wexler, D. L., Kuramitsu, H. & Bowen, W. H. (1996). Cariogenicity of *Streptococcus mutans* strains with defects in fructan metabolism assessed in a program-fed specific-pathogen-free rat model. *J Dent Res* **75**, 1572-1577.
Burne, R. A., Chen, Y. Y. & Penders, J. E. (1997). Analysis of gene expression in *Streptococcus mutans* in biofilms in vitro. *Adv Dent Res* **11**, 100-109.
Butcher, R. A., Bhullar, B. S., Perlstein, E. O., Marsischky, G., LaBaer, J. & Schreiber, S. L. (2006). Microarray-based method for monitoring yeast overexpression strains reveals small-molecule targets in TOR pathway. *Nat Chem Biol* **2**, 103-109.
Calmes, R. (1978). Involvement of phosphoenolpyruvate in the catabolism of caries-conducive disaccharides by *Streptococcus mutans*: lactose transport. *Infect Immun* **19**, 934–942.
Calmes, R. & Brown, A. T. (1979). Regulation of lactose catabolism in *Streptococcus mutans*: purification and regulatory properties of phospho-beta-galactosidase. *Infect Immun* **23**, 68-79.
Catt, D. M. & Gregory, R. L. (2005). *Streptococcus mutans* murein hydrolase. *J Bacteriol* **187**, 7863-7865.
Caufield, P. W., Li, Y., Dasanayake, A. & Saxena, D. (2007). Diversity of lactobacilli in the oral cavities of young women with dental caries. *Caries Res* **41**, 2-8.
Chandramohan, L., Ahn, J.-S., Weaver, K. E. & Bayles, K. W. (2009). An overlap between the control of programmed cell death in *Bacillus anthracis* and sporulation. *J Bacteriol* **191**, 4103-4110.
Chee, M., Yang, R., Hubbell, E. & other authors (1996). Accessing genetic information with high-density DNA arrays. *Science* **274**, 610-614.
Clarke, J. K. (1924). On the bacterial factor in the etiology of dental caries. *Brit J Exp Pathol* **5**, 141–147.
Collins, D. M., Gabric, D. M. & De Lisle, G. W. (1989). Identification of a repetitive DNA sequence specific to *Mycobacterium paratuberculosis*. *FEMS Microbiol Lett* **51,** 175-178.
Cook, K. L. & Sayler, G. S. (2003). Environmental application of array technology: promise, problems and practicalities. *Curr Opin Biotechnol* **14**, 311-318.
Coppée, J.-Y. (2008). Do DNA microarrays have their future behind them? *Microbes Infect* **10**, 1067-1071.
Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annu Rev Microbiol* **49**, 711-745.

Cote, C. K., Cvitkovitch, D., Bleiweis, A. S. & Honeyman, A. L. (2000). A novel β-glucoside-specific PTS locus from *Streptococcus mutans* that is not inhibited by glucose. *Microbiol* **146**, 1555-1563.
Cvitkovitch, D. G. (2001). Genetic competence and transformation in oral streptococci. *Crit Rev Oral Biol Med* **12**, 217-243.
Dashper, S. G. & Reynolds, E. C. (1992). pH regulation by *Streptococcus mutans*. *J Dent Res* **71**, 1159-1165.
Dashper, S. G. & Reynolds, E. C. (2000). Effects of organic acid anions on growth, glycolysis, and intracellular pH of oral streptococci. *J Dent Res* **79**, 90-96.
de Jong, A., van Hijum, S. A. F. T., Bijlsma, J. J. E., Kok, J. & Kuipers, O. P. (2006). BAGEL: a web-based bacteriocin genome mining tool. *Nucl Acids Res* **34**, W273-279.
de Soet, J. J., Nyvad, B. & Kilian, M. (2000). Strain-related acid production by oral streptococci. *Caries Res* **34**, 486-490.
Deng, D. M., Liu, M. J., ten Cate, J. M. & Crielaard, W. (2007). The VicRK system of *Streptococcus mutans* responds to oxidative stress. *J Dent Res* **86**, 606-610.
Dirix, G., Monsieurs, P., Marchal, K., Vanderleyden, J. & Michiels, J. (2004). Screening genomes of Gram-positive bacteria for double-glycine-motif-containing peptides. *Microbiol* **150**, 1121-1126.
Dresen, I. M., Hüsing, J., Kruse, E., Boes, T. & Jöckel, K. H. (2003). Software packages for quantitative microarray-based gene expression analysis. *Curr Pharm Biotechnol* **4**, 417-437.
Dubrac, S., Bisicchia, P., Devine, K. M. & Msadek, T. (2008). A matter of life and death: cell wall homeostasis and the WalKR (YycGF) essential signal transduction pathway. *Mol Microbiol* **70**, 1307-1322.
Echenique, J., Kadioglu, A., Romao, S., Andrew, P. W. & Trombe, M.-C. (2004). Protein serine/threonine kinase StkP positively controls virulence and competence in *Streptococcus pneumoniae*. *Infect Immun* **72**, 2434-2437.
Eckmann, L., Smith, J. R., Housley, M. P., Dwinell, M. B. & Kagnoff, M. F. (2000). Analysis by high density cDNA arrays of altered gene expression in human intestinal epithelial cells in response to infection with the invasive enteric bacteria *Salmonella*. *J Biol Chem* **275**, 14084-14094.
Eisen, M. B., Spellman, P. T., Brown, P. O. & Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* **95**, 14863-14868.
Ellwood, D. C., Phipps, P. J. & Hamilton, I. R. (1979). Effect of growth rate and glucose concentration on the activity of the phosphoenolpyruvate phosphotransferase system in *Streptococcus mutans* Ingbritt grown in continuous culture. *Infect Immun* **23**, 224-231.
Fejerskov, O. (2004). Changing paradigms in concepts on dental caries: consequences for oral health care. *Caries Res* **38**, 182-191.
Fitzgerald, R. J. & McDaniel, E. G. (1960). Dental calculus in the germ-free rat. *Arch Oral Biol* **2**, 239-240.
Foster, J. W. & Hall, H. K. (1991). Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium*. J Bacteriol **173**, 5129-5135.
Freiberg, C., Fischer, H. P. & Brunner, N. A. (2005). Discovering the mechanism of action of novel antibacterial agents through transcriptional profiling of conditional mutants. *Antimicrob Agents Chemother* **49**, 749-759.
Gauthier, L., Mayrand, D. & Vadeboncoeur, C. (1984). Isolation of a novel protein involved in the transport of fructose by an inducible phosphoenolpyruvate fructose phosphotransferase system in *Streptococcus mutans*. *J Bacteriol* **160**, 755–763.

Gawron-Burke, C. & Clewell, D. B. (1984). Regeneration of insertionally inactivated streptococcal DNA fragments after excision of transposon Tn*916* in *Escherichia coli*: strategy for targeting and cloning of genes from gram-positive bacteria. *J Bacteriol* **159**, 214-221

Gibbons, R. J., Berman, K. S., Koettner, P. & Kapsimalis, B. (1966). Dental caries and alveolar bone loss in gnotobiotic rats infected with capsule-forming streptococci of human origin. *Arch Oral Biol* **11**, 549-560.

Gmür, R. & Guggenheim, B. (1983). Antigenic heterogeneity of *Bacteroides intermedius* as recognized by monoclonal antibodies. *Infect Immun* **42**, 459-470.

Groicher, K. H., Firek, B. A., Fujimoto, D. F. & Bayles, K. W. (2000). The *Staphylococcus aureus lrgAB* operon modulates murein hydrolase activity and penicillin tolerance. *J Bacteriol* **182**, 1794-1801.

Guggenheim, B., Giertsen, E., Schüpbach, P. & Shapiro, S. (2001a). Validation of an *in vitro* biofilm model of supragingival plaque. *J Dent Res* **80**, 363-370.

Guggenheim, M., Shapiro, S., Gmür, R. & Guggenheim, B. (2001b). Spatial arrangements and associative behavior of species in an in vitro oral biofilm model. *Appl Environ Microbiol* **67**, 1343-1350.

Hahn, K., Faustoferri, R. C. & Quivey, R. G., Jr. (1999). Induction of an AP endonuclease activity in *Streptococcus mutans* during growth at low pH. *Mol Microbiol* **31**, 1489-1498.

Hamilton, I. R. & Lebtag, H. (1979). Lactose metabolism by *Streptococcus mutans*: evidence for induction of the tagatose 6-phosphate pathway. *J Bacteriol* **140**, 1102-1104.

Hartke, A., Giard, J.-C., Laplace, J.-M. & Auffray, Y. (1998). Survival of *Enterococcus faecalis* in an oligotrophic microcosm: changes in morphology, development of general stress resistance, and analysis of protein synthesis. *Appl Environ Microbiol* **64**, 4238-4245.

Herbert, K. C. & Foster, S. J. (2001). Starvation survival in *Listeria monocytogenes*: characterization of the response and the role of known and novel components. *Microbiol* **147**, 2275-2284.

Herrero, A. A., Gomez, R. F., Snedecor, B., Tolman, C. J. & Roberts, M. F. (1985). Growth inhibition of *Clostridium thermocellum* by carboxylic acids: a mechanism based on uncoupling by weak acids. *Appl Microbiol Biotechnol* **22**, 53-62.

Higgins, C. F. (2001). ABC transporters: physiology, structure and mechanism - an overview. *Res Microbiol* **152**, 205-210.

Higuchi, M., Yamamoto, Y., Poole, L. B., Shimada, M., Sato, Y., Takahashi, N. & Kamio, Y. (1999). Functions of two types of NADH oxidases in energy metabolism and oxidative stress of *Streptococcus mutans*. *J Bacteriol* **181**, 5940-5947.

Hossain, H., Tchatalbachev, S. & Chakraborty, T. (2006). Host gene expression profiling in pathogen-host interactions. *Current Opinion in Immunology* **18**, 422-429.

Hussain, H., Branny, P. & Allan, E. (2006). A eukaryotic-type serine/threonine protein kinase is required for biofilm formation, genetic competence, and acid resistance in *Streptococcus mutans*. *J Bacteriol* **188**, 1628-1632.

Huyghe, A., Francois, P., Charbonnier, Y. & other authors (2008). Novel microarray design strategy to study complex bacterial communities. *Appl Environ Microbiol* **74**, 1876-1885.

Jacobson, G. R., Poy, F. & Lengeler, J. W. (1990). Inhibition of *Streptococcus mutans* by the antibiotic streptozotocin: mechanisms of uptake and the selection of carbohydrate-negative mutants. *Infect Immun* **58**, 543-549.

Jenkinson, H. F. & Demuth, D. R. (1997). Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. *Mol Microbiol* **23**, 183-190.

Jin, H. & Pancholi, V. (2006). Identification and biochemical characterization of a eukaryotic-type serine/threonine kinase and its cognate phosphatase in *Streptococcus pyogenes*: their biological

functions and substrate identification. *J Mol Biol* **357**, 1351-1372.
Jung Lin, W.-J., Walthers, D., Connelly, J. E., Burnside, K., Jewell, K. A., Kenney, L. J. & Rajagopal, L. (2009). Threonine phosphorylation prevents promoter DNA binding of the Group B Streptococcus response regulator CovR. *Mol Microbiol* **71**, 1477-1495.

Kawada-Matsuo, M., Shibata, Y. & Yamashita, Y. (2009). Role of two component signaling response regulators in acid tolerance of *Streptococcus mutans*. *Oral Microbiol Immunol* **24**, 173-176.
Kenney, A. C. & Cole, J. A. (1983). Identification of a 1,3-glucosyltransferase involved in insoluble glucan synthesis by serotype c strains of *Streptococcus mutans*. *FEMS Microbiol Lett* **16**, 159-162.
Kilic, A. O., Tao, L., Zhang, Y., Lei, Y., Khammanivong, A. & Herzberg, M. C. (2004). Involvement of *Streptococcus gordonii* beta-glucoside metabolism systems in adhesion, biofilm formation, and in vivo gene expression. *J Bacteriol* **186**, 4246-4253.
Kishimoto, E., Hay, D. I. & Gibbons, R. J. (1989). A human salivary protein which promotes adhesion of *Streptococcus mutans* serotype c strains to hydroxyapatite. *Infect Immun* **57**, 3702-3707.
Kostic, T., Weilharter, A., Rubino, S., Delogu, G., Uzzau, S., Rudi, K., Sessitsch, A. & Bodrossy, L. (2007). A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of nonpathogens. *Anal Biochem* **360**, 244-254.
Kreth, J., Hung, D. C. I., Merritt, J., Perry, J., Zhu, L., Goodman, S. D., Cvitkovitch, D. G., Shi, W. & Qi, F. (2007). The response regulator ComE in Streptococcus mutans functions both as a transcription activator of mutacin production and repressor of CSP biosynthesis. *Microbiol* **153**, 1799-1807.
Kreth, J., Merritt, J. & Qi, F. (2009). Bacterial and host interactions of oral streptococci. *DNA Cell Biol* **28**, 397-403.
Kuramitsu, H. K. (1993). Virulence factors of mutans streptococci: role of molecular genetics. *Crit Rev Oral Biol Med* **4**, 159-176.
Lamont, J. R., Burne, R. A., Lantz, M. S. & Leblanc, D. J. (2006). *Oral microbiology and immunology*. Washington DC.: ASM Press.
Lappin-Scott, H. M. & Bass, C. (2001). Biofilm formation: Attachment, growth, and detachment of microbes from surfaces. *Am J of Infect Control* **29**, 250-251.
Lemos, J. A., Abranches, J. & Burne, R. A. (2005). Responses of cariogenic streptococci to environmental stresses. *Curr Issues Mol Biol* **7**, 95-107.
Lemos, J. A. & Burne, R. A. (2008). A model of efficiency: stress tolerance by *Streptococcus mutans*. *Microbiol* **154**, 3247-3255.
Lemos, J. A., Nascimento, M. M., Lin, V. K., Abranches, J. & Burne, R. A. (2008). Global regulation by (p)ppGpp and CodY in *Streptococcus mutans*. *J Bacteriol* **190**, 5291-5299.
Levesque, C. M., Mair, R. W., Perry, J. A., Lau, P. C. Y., Li, Y. H. & Cvitkovitch, D. G. (2007). Systemic inactivation and phenotypic characterization of two-component systems in expression of *Streptococcus mutans* virulence properties. *Lett Appl Microbiol* **45**, 398-404.
Li, Y., Ge, Y., Saxena, D. & Caufield, P. W. (2007). Genetic profiling of the oral microbiota associated with severe early-childhood caries. *J Clin Microbiol* **45**, 81-87.
Li, Y. H., Hanna H.M, Svensater, G., Ellen, R. P. & Cvitkovitch, D. G. (2001a). Cell density modulates acid adaptation in *Streptococcus mutans*: implications for survival in biofilms. *J Bacteriol* **183**, 6875-6884.
Li, Y. H., Lau, P. C., Lee, J. H., Ellen, R. P. & Cvitkovitch, D. G. (2001b). Natural genetic transformation of *Streptococcus mutans* growing in biofilms. *J Bacteriol* **183**, 897-908.
Li, Y. H., Lau, P. C., Tang, N., Svensater, G., Ellen, R. P. & Cvitkovitch, D. G. (2002a). Novel two-component regulatory system involved in biofilm formation and acid resistance in *Streptococcus*

mutans. *J Bacteriol* **184**, 6333-6342.
Li, Y. H., Tang, N., Aspiras, M. B., Lau, P. C., Lee, J. H., Ellen, R. P. & Cvitkovitch, D. G. (2002b). A quorum-sensing signaling system essential for genetic competence in *Streptococcus mutans* is involved in biofilm formation. *J Bacteriol* **184**, 2699-2708.
Liberman, E. S. & Bleiweis, A. S. (1984). Role of the phosphoenolpyruvate-dependent glucose phosphotransferase system of *Streptococcus mutans* GS5 in the regulation of lactose uptake. *Infect Immun* **43**, 536-542.
Lo, A., Seers, C., Boyce, J., Dashper, S., Slakeski, N., Lissel, J. P. & Reynolds, E. (2009). Comparative transcriptomic analysis of *Porphyromonas gingivalis* biofilm and planktonic cells. *BMC Microbiol* **9**, 18-28.
Lodge, J. & Jacobson, G. R. (1988). Starvation-induced stimulation of sugar uptake in *Streptococcus mutans* is due to an effect on the activities of preexisting proteins of the phosphotransferase system. *Infect Immun* **56**, 2594-2600.
Loimaranta, V., Jakubovics, N. S., Hytonen, J., Finne, J., Jenkinson, H. F. & Stromberg, N. (2005). Fluid- or surface-phase human salivary scavenger protein gp340 exposes different bacterial recognition properties. *Infect Immun* **73**, 2245-2252.
Loo, C. Y., Mitrakul, K., Voss, I. B., Hughes, C. V. & Ganeshkumar, N. (2003). Involvement of an inducible fructose phosphotransferase operon in *Streptococcus gordonii* biofilm formation. *J Bacteriol* **185**, 6241-6254.
Madiraju, M. V., Brunner, D. P. & Wilkinson, B. J. (1987). Effects of temperature, NaCl, and methicillin on penicillin-binding proteins, growth, peptidoglycan synthesis, and autolysis in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **31**, 1727–1733.
Magnuson, R. D. (2007). Hypothetical functions of toxin-antitoxin systems. *J Bacteriol* **189**, 6089-6092.
Mark, L. & Errington, J. (2005). Roles for MreC and MreD proteins in helical growth of the cylindrical cell wall in *Bacillus subtilis*. *Mol Microbiol* **57**, 1196-1209.
Marsh, P. D. (2004). Dental plaque as a microbial biofilm. *Caries Res* **38**, 204-211.
Maryanski, J. H. & Wittenberger, C. L. (1975). Mannitol transport in *Streptococcus mutans*. *J Bacteriol* **124**, 1475–1481.
Matsumoto-Nakano, M. & Kuramitsu, H. K. (2006). Role of bacteriocin immunity proteins in the antimicrobial sensitivity of *Streptococcus mutans*. *J Bacteriol* **188**, 8095-8102.
Matsumura, M., Izumi, T., Matsumoto, M., Tsuji, M., Fujiwara, T. & Ooshima, T. (2003). The role of glucan-binding proteins in the cariogenicity of *Streptococcus mutans*. *Microbiol Immunol* **47**, 213-215.
Mattos-Graner, R. O., Jin, S., King, W. F., Chen, T., Smith, D. J. & Duncan, M. J. (2001). Cloning of the *Streptococcus mutans* gene encoding glucan binding protein B and analysis of genetic diversity and protein production in clinical isolates. *Infect Immun* **69**, 6931-6941.
McNeill, K. & Hamilton, I. R. (2004). Effect of acid stress on the physiology of biofilm cells of *Streptococcus mutans*. *Microbiol* **150**, 735-742.
Merritt, J., Qi, F., Goodman, S. D., Anderson, M. H. & Shi, W. (2003). Mutation of *luxS* affects biofilm formation in *Streptococcus mutans*. *Infect Immun* **71**, 1972-1979.
Merritt, J., Kreth, J., Shi, W. & Qi, F. (2005a). LuxS controls bacteriocin production in *Streptococcus mutans* through a novel regulatory component. *Mol Microbiol* **57**, 960-969.
Merritt, J., Qi, F. & Shi, W. (2005b). A unique nine-gene *comY* operon in *Streptococcus mutans*. *Microbiol* **151**, 157-166.
Mimura, C. S., Eisenberg, L. B. & Jacobson, G. R. (1984). Resolution of the phosphotransferase enzymes of Streptococcus mutans: purification and preliminary characterization of a heat-stable

phosphocarrier protein. *Infect Immun* **44**, 708-715.
Mortlock, R. P. (1992). The evolution of metabolic function. CRC Press, Inc 2000 Corporate Blvd., N.W., Boca Raton, Florida.
My-Van La, D. R. P. R. (2008). Regulation of whole bacterial pathogen transcription within infected hosts. *FEMS Microbiol Rev* **32**, 440-460.
Néron, S. & Vadeboncoeur, C. (1987a). Two functionally different glucose phosphotransferase transport systems in *Streptococcus mutans* and *Streptococcus sobrinus*. *Oral Microbiol Immunol* **2**, 171-177.
Néron, S. & Vadeboncoeur, C. (1987b). Evidence for the presence of two distinct phosphoenolpyruvate: mannose phosphotransferase systems in *Streptococcus mutans* GS5-2. *FEMS Microbiol Lett* **42**, 7-11.
Ng, W. L., Robertson, G. T., Kazmierczak, K. M., Zhao, J., Gilmour, R. & Winkler, M. E. (2003). Constitutive expression of PcsB suppresses the requirement for the essential VicR (YycF) response regulator in *Streptococcus pneumoniae* R6. *Mol Microbiol* **50**, 1647-1663.
Ng, W. L., Kazmierczak, K. M. & Winkler, M. E. (2004). Defective cell wall synthesis in *Streptococcus pneumoniae* R6 depleted for the essential PcsB putative murein hydrolase or the VicR (YycF) response regulator. *Mol Microbiol* **53**, 1161-1175.
Nguyen, P. T., Abranches, J., Phan, T. N. & Marquis, R. E. (2002). Repressed respiration of oral streptococci grown in biofilms. *Curr Microbiol* **44**, 262-266.
Nováková, L., Sasková, L., Pallová, P., Janeček, J., Novotná, J., Ulrych, A., Echenique, J., Trombe, M.-C. & Branny, P. (2005). Characterization of a eukaryotic type serine/threonine protein kinase and protein phosphatase of *Streptococcus pneumoniae* and identification of kinase substrates. *FEBS J* **272**, 1243-1254.
Orland , F. J. (1959). A review of dental research using germfree animals. *Ann N Y Acad Sci* **78**, 285-289.
Paik, S., Brown, A., Munro, C. L., Cornelissen, C. N. & Kitten, T. (2003). The *sloABCR o*peron of *Streptococcus mutans* encodes an Mn and Fe transport system required for endocarditis virulence and its Mn-dependent repressor. *J Bacteriol* **185**, 5967-5975.
Park, T., Struck, D. K., Deaton, J. F. & Young, R. (2006). Topological dynamics of holins in programmed bacterial lysis. *Proc Natl Acad Sci U S A* **103**, 19713-19718.
Patton, T. G., Yang, S.-J. & Bayles, K. W. (2006). The role of proton motive force in expression of the *Staphylococcus aureus cid* and *lrg* operons. *Mol Microbiol* **59**, 1395-1404.
Perry, J. A., Lévesque, C. M., Suntharaligam, P., Mair, R. W., Bu, M., Cline, R. T., Peterson, S. N. & Cvitkovitch, D. G. (2008). Involvement of *Streptococcus mutans* regulator RR11 in oxidative stress response during biofilm growth and in the development of genetic competence. *Lett in Appl Microbiol* **47**, 439-444.
Perry, J. A., Cvitkovitch, D. G. & Céline, M. L., C.M. (2009). Cell death in *Streptococcus mutans* biofilms: a link between CSP and extracellular DNA. *FEMS Microbiol Lett* **299**, 261-266.
Petersen, F. C., Tao, L. & Scheie, A. A. (2005). DNA binding-uptake system: a link between cell-to-cell communication and biofilm formation. *J Bacteriol* **187**, 4392-4400.
Podbielski, A. & Kreikemeyer, B. (2004). Cell density-dependent regulation: basic principles and effects on the virulence of Gram-positive cocci. *Int J Infect Dis* **8**, 81-95.
Poy, F. & Jacobson, G. R. (1990). Evidence that a low-affinity sucrose phosphotransferase activity in *Streptococcus mutans* GS-5 is a high-affinity trehalose uptake system. *Infect Immun* **58**, 1479-1480.
Qi, F., Chen, P. & Caufield, P. W. (1999). Purification of mutacin III from group III *Streptococcus mutans* UA787 and genetic analyses of mutacin III biosynthesis genes. *Appl Environ Microbiol* **65**, 3880-3887.

Qi, F., Chen, P. & Caufield, P. W. (2001). The group I strain of *Streptococcus mutans*, UA140, produces both the lantibiotic mutacin I and a nonlantibiotic bacteriocin, mutacin IV. *Appl Environ Microbiol* **67**, 15-21.
Qi, F., Merritt, J., Lux, R. & Shi, W. (2004). Inactivation of the *ciaH g*ene in *Streptococcus mutans* diminishes mutacin production and competence development, alters sucrose-dependent biofilm formation, and reduces stress tolerance. *Infect Immun* **72**, 4895-4899.
Quivey, R. G., Kuhnert, W. L. & Hahn, K. (2001). Genetics of acid adaptation in oral streptococci. *Crit Rev Oral Biol Med* **12**, 301-314.
Rajagopal, L., Clancy, A. & Rubens, C. E. (2003). A eukaryotic type serine/threonine kinase and phosphatase in *Streptococcus agalactiae* reversibly phosphorylate an inorganic pyrophosphatase and affect growth, cell segregation, and virulence. *J Biol Chem* **278**, 14429-14441.
Rajagopal, L., Silvestroni, A., Vo, A. & Rubens, C. E. (2006). Regulation of cytotoxin expression by converging eukaryotic-type and two-component signalling mechanisms in *Streptococcus agalactiae*. *Mol Microbiol* **62**, 941-957.
Rathsam, C., Eaton, R. E., Simpson, C. L., Browne, G. V., Berg, T., Harty, D. W. S. & Jacques, N. A. (2005a). Up-regulation of competence- but not stress-responsive proteins accompanies an altered metabolic phenotype in *Streptococcus mutans* biofilms. *Microbiol* **151**, 1823-1837.
Rathsam, C., Eaton, R. E., Simpson, C. L., Browne, G. V., Valova, V. A., Harty, D. W. S. & Jacques, N. A. (2005b). Two-dimensional fluorescence difference gel electrophoretic analysis of *Streptococcus mutans* biofilms. *J Proteome Res* **4**, 2161-2173.
Rice, K. C., Firek, B. A., Nelson, J. B., Yang, S.-J., Patton, T. G. & Bayles, K. W. (2003). The *Staphylococcus aureus cidAB* operon: evaluation of its role in regulation of murein hydrolase activity and penicillin tolerance. *J Bacteriol* **185**, 2635-2643.
Rice, K. C., Mann, E. E., Endres, J. L., Weiss, E. C., Cassat, J. E., Smeltzer, M. S. & Bayles, K. W. (2007). The *cidA* murein hydrolase regulator contributes to DNA release and biofilm development in *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* **104**, 8113-8118.
Rogers, A. H. (1976). Bacteriocinogeny and the properties of some bacteriocins of *Streptococcus mutans*. *Arch Oral Biol* **21**, 99-104.
Rosan, B. & Lamont, R. J. (2000). Dental plaque formation. *Microbes Infect* **2**, 1599-1607.
Rosenberger, C. M., Scott, M. G., Gold, M. R., Hancock, R. E. W. & Finlay, B. B. (2000). *Salmonella typhimurium* infection and lipopolysaccharide stimulation induce similar changes in macrophage gene expression. *J Immunol* **164**, 5894-5904.
Russell, R. R., Aduse-Opoku, J., Sutcliffe, I. C., Tao, L. & Ferretti, J. J. (1992). A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. *J Biol Chem* **267**, 4631-4637.
Sablon, E., Contreras, B. & Vandamme, E. (2000). Antimicrobial peptides of lactic acid bacteria: mode of action, genetics and biosynthesis. In *New Products and New Areas of Bioprocess Engineering*, 21-60.
Samaranayake, L. P. (2002). Microbiology of dental caries. In *Essential Microbiology for dentistry*, 217-223. London: Churchill Livingstone.
Sasková, L., Novaková, L., Basler, M. & Branny, P. (2007). Eukaryotic-type serine/threonine protein kinase StkP is a global regulator of gene expression in *Streptococcus pneumoniae*. *J Bacteriol* **189**, 4168-4179.
Schachtele, C. F. & Mayo, J. A. (1973). Phosphoenolpyruvate-dependent glucose transport in oral Streptococci. *J Dent Res* **52**, 1209-1215.
Scheie, A. A. & Petersen, F. C. (2004). The biofilm concept: consequences for future prophylaxis of oral diseases? *Crit Rev Oral Biol Med* **15**, 4-12.

Schena, M., Shalon, D., Davis, R. W. & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467-470.
Selwitz, R. H., Ismail, A. I. & Pitts, N. B. (2007). Dental caries. *The Lancet* **369**, 51-59.
Senadheera, D., Lee, A. W. C., Hung, D. C. & Cvitkovitch, D.G. (2007a). *Streptococcus mutans* quorum-sensing system controls *gtfB/C/D*, *ftf* and *gbpB* expression. *General meeting hosted by the American Society for Microbiology Quebec, Canada*.
Senadheera, D., Mair, R. & Cvitkovitch, D. G. (2007b). *Streptococcus mutans* competence stimulated peptide affects *gtfB/C* and *vicR/K* expression and biofilm structure. *Biofilm conference hosted by the American Society for Microbiology, Quebec, Canada*
Senadheera, D., Krastel, K., Mair, R., Persadmehr, A., Abranches, J., Burne, R. & Cvitkovitch, D. G. (2009). Inactivation of *VicK* affects acid production and acid survival of Streptococcus mutans. *J Bacteriol*, JB.00793-00709.
Senadheera, M. D., Guggenheim, B., Spatafora, G. A. & other authors (2005). A *VicRK* signal transduction system in *Streptococcus mutans* affects *gtfBCD*, *gbpB*, and *ftf* expression, biofilm formation, and genetic competence development. *J Bacteriol* **187**, 4064-4076.
Senadheera, M. D., Lee, A. W. C., Hung, D. C. I., Spatafora, G. A., Goodman, S. D. & Cvitkovitch, D. G. (2007c). The *Streptococcus mutans vicX* Gene product modulates *gtfB*/*C* expression, biofilm formation, genetic competence, and oxidative stress tolerance. *J Bacteriol* **189**, 1451-1458.
Shah, I. M., Laaberki, M.-H., Popham, D. L. & Dworkin, J. (2008). A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486-496.
Shemesh, M., Tam, A. & Steinberg, D. (2007a). Expression of biofilm-associated genes of *Streptococcus mutans* in response to glucose and sucrose. *J Med Microbiol* **56**, 1528-1535.
Shemesh, M., Tam, A. & Steinberg, D. (2007b). Differential gene expression profiling of *Streptococcus mutans* cultured under biofilm and planktonic conditions. *Microbiol* **153**, 1307-1317.
Simpson, C. L. & Russell, R. R. B. (1998). Intracellular alpha -amylase of *Streptococcus mutans*. *J Bacteriol* **180**, 4711-4717.
Slee, A. M. & Tanzer, J. M. (1979a). Phosphoenolpyruvate-dependent sucrose phosphotransferase activity in *Streptococcus mutans* NCTC 10449. *Infect Immun* **24**, 821-828.
Slee, A. M. & Tanzer, J. M. (1979b). Phosphoenolpyruvate-dependent sucrose phosphotransferase activity in five serotypes of *Streptococcus mutans*. *Infect Immun* **26**, 783-786.
Slee, A. M. & Tanzer, J. M. (1983). The repressible metabolism of sorbitol (D-glucitol) by intact cells of the oral plaque-forming bacterium *Streptococcus mutans*. *Arch Oral Biol 28(9)*, 839-845.
Soufi, B., Gnad, F., Jensen, P. R., Petranovic, D., Mann, M., Mijakovic, I. & Macek, B. (2008). The Ser/Thr/Tyr phosphoproteome of *Lactococcus lactis* IL1403 reveals multiply phosphorylated proteins. *Proteomic* **8**, 3486-3493.
St Martin, E. J. & Wittenberger, C. L. (1979). Characterization of a phosphoenolpyruvate-dependent sucrose phosphotransferase system in Streptococcus mutans. *Infect Immun* **24**, 865-868.
Starke, E. M. L., Smoot, J. C., Smoot, L. M., Liu, W.-T., Chandler, D. P., Lee, H. H. & Stahl, D. A. (2006). Technology development to explore the relationship between oral health and the oral microbial community. *BMC Oral Health* **8**, 81-95.
Suntharalingam, P., Senadheera, M. D., Mair, R. W., Levesque, C. M. & Cvitkovitch, D. G. (2009). The *LiaFSR* system regulates the cell envelope stress response in *Streptococcus mutans*. *J Bacteriol* **191**, 2973-2984.
Sztajer, H., Lemme, A., Vilchez, R., Schulz, S., Geffers, R., Yip, C. Y. Y., Levesque, C. M., Cvitkovitch, D. G. & Wagner-Dobler, I. (2008). Autoinducer-2-regulated genes in *Streptococcus mutans* UA159 and global metabolic effect of the *luxS* mutation. *J Bacteriol* **190**, 401-415.

Tandler, B. (1990). Improved uranyl acetate staining for electron microscopy. *J Electron Microsc Tech* **16***,* 81-82

ten Cate, J. M. (2006). Biofilms, a new approach to the microbiology of dental plaque. *Odontology* **94**, 1-9.

Thedei, G. J., Leitão, D. P. S., Bolean, M., Paulino, T. P., Spadaro, A. C. C. & Ciancaglini, P. (2008). Toluene permeabilization differentially affects F- and P-type ATPase activities present in the plasma membrane of *Streptococcus mutans*. *Braz J Med Biol Res* **41**, 1047-1053.

Thibessard, A., Borges, F., Fernandez, A., Gintz, B., Decaris, B. & Leblond-Bourget, N. (2004). Identification of *Streptococcus thermophilus* CNRZ368 genes involved in defense against superoxide stress. *Appl Environ Microbiol* **70**, 2220-2229.

Thurnheer, T., Gmür, R. & Guggenheim, B. (2004). Multiplex FISH analysis of a six-species bacterial biofilms. *J Microbiol Methods* **56**, 37-47.

Trahan, L., Bareil, M., Gauthier, L. & Vadeboncoeur, C. (1985). Transport and phosphorylation of xylitol by a fructose phosphotransferase system in S*treptococcus mutans*. *Caries Res* **19**, 53-63.

Vadeboncoeur, C. & Proulx, M. (1984). Lactose transport in *Streptococcus mutans*: isolation and characterization of factor III^{lac}, a specific protein component of the phosphoenolpyruvate-lactose phosphotransferase system. *Infect Immun* **46**, 213-219.

Vadeboncoeur, C. & Pelletier, M. (1997). The phosphoenolpyruvate:sugar phosphotransferase system of oral streptococci and its role in the control of sugar metabolism. *FEMS Microbiol Rev* **19**, 187-207.

van der Ploeg, J. R. & Guggenheim, B. (2004). Deletion of *gtfC* of *Streptococcus mutans* has no influence on the composition of a mixed-species *in vitro* biofilm model of supragingival plaque. *Eur J Oral Sci* **112**, 433-438.

van der Ploeg, J. R. (2005). Regulation of bacteriocin production in *Streptococcus mutans* by the quorum-sensing system required for development of genetic competence. *J Bacteriol* **187**, 3980-3989.

van der Ploeg, J. R. (2009). Analysis of CRISPR in *Streptococcus mutans* suggests frequent occurrence of acquired immunity against infection by M102-like bacteriophages. *Microbiol* **155,** 1966-1976.

Venable, J. H. & Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J Cell Biol* **25**, 407-408.

Vianna, M. E., H.P., H., Gomes, B. P. F. A. & Conrads, G. (2005). Microarrays complement culture methods for identification of bacteria in endodontic infections. *Oral Microbiol Immunol* **20**, 253-258.

Wagner, C., Saizieu Ad, A., Schonfeld, H. J., Kamber, M., Lange, R., Thompson, C. J. & Page, M. G. (2002). Genetic analysis and functional characterization of the *Streptococcus pneumoniae vic* operon. *Infect Immun* **70**, 6121-6128.

Wang, B. & Kuramitsu, H. K. (2003). Control of enzyme II^{scr} and sucrose-6-phosphate hydrolase activities in *Streptococcus mutans* by transcriptional repressor ScrR binding to the *cis*-active determinants of the *scr* regulon. *J Bacteriol* **185**, 5791-5799.

Wang, I. N., Smith, D. L. & Young, R. (2000). Holins: the protein clocks of bacteriophage infections. *Annu Rev Microbiol* **54**, 799-825.

Welin, J., Wilkins, J. C., Beighton, D. & Svensater, G. (2004). Protein expression by *Streptococcus mutans* during initial stage of biofilm formation. *Appl Environ Microbiol* **70**, 3736-3741.

Wen, Z. T., Baker, H. V. & Burne, R. A. (2006). Influence of BrpA on critical virulence attributes of *Streptococcus mutans*. *J Bacteriol* **188**, 2983-2992.

Woo, Y., Affourtit, J., Daigle, S., Viale, A., Johnson, K., Naggert, J. & Churchill, G. (2004). A comparison of cDNA, oligonucleotide, and Affymetrix GeneChip gene expression microarray platforms. *J Biomol Tech* **15**, 276-284.

Wood, T. K. (2009). Insights on *Escherichia coli* biofilm formation and inhibition from whole-

transcriptome profiling. *Environ Microbiol* **11**, 1-15.
Würsch, P. & Koellreutter, B. (1985). Maltotriitol inhibition of maltose metabolism in *Streptococcus mutans* via maltose transport, amylomaltase and phospho-alpha-glucosidase activities. *Caries Res* **19**, 439-449.
Xiang, C., Young, H., Alterson, H. & other authors (1999). Comparison of cellular gene expression in Ebola-Zaire and Ebola-Reston virus-infected primary human monocytes. *Nat Genetics* **23**, 82-82.
Yang, S.-J., Rice, K. C., Brown, R. J., Patton, T. G., Liou, L. E., Park, Y. H. & Bayles, K. W. (2005). A LysR-type regulator, CidR, is required for induction of the *Staphylococcus aureus cidABC* operon. *J Bacteriol* **187**, 5893-5900.
Yeats, C., Finn, R. D. & Bateman, A. (2002). The PASTA domain: a β-lactam-binding domain. *Trends Biochem Sci* **27**, 438-440.

Appendix

Table 1. Differentially expressed genes in biofilms *vs.* planktonically grown cells in stationary phase (p-value<0.05). Ratio represents the value for each differentially expressed gene in biofilms.

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.575c	putative membrane protein	lrgA	62.15	5.19E-03
SMU.574c	putative membrane protein	lrgB	15.72	3.63E-04
SMU.609	putative 40K cell wall protein precursor		14.23	2.19E-03
SMU.173	putative ppGpp-regulated growth inhibitor	ppGpp	10.14	1.08E-04
SMU.172	hypothetical protein; putative cell growth regulatory protein		8.44	6.95E-04
SMU.179	hypothetical protein		7.55	3.01E-04
SMU.1976c	hypothetical protein		7.31	3.22E-03
SMU.500	putative ribosome-associated protein		6.49	2.41E-04
SMU.1975c	hypothetical protein; possible membrane protein		6.27	3.19E-04
SMU.494	fructose-6-phosphate aldolase		5.96	9.36E-04
SMU.426	copper-transporting ATPase; P-type ATPase	copA	5.96	1.64E-03
SMU.1116c	hypothetical protein		5.34	4.28E-03
SMU.448	hypothetical protein		5.26	7.14E-03
SMU.956	putative Clp-like ATP-dependent protease, ATP-binding subunit	clp	5.06	3.47E-02
SMU.441	putative transcriptional regulator		4.86	3.95E-06
SMU.424	negative transcriptional regulator	copY	4.63	4.22E-03
SMU.1674	putative aminotransferase; probable beta-cystathionase	metC	4.5	1.98E-04
SMU.442	conserved hypothetical protein		4.27	7.74E-05
SMU.34	putative phosphoribosylformylglycinamide cyclo-ligase (AIRS) phosphoribosyl aminoimidazole synthetase	purM	4.16	7.15E-03
SMU.32	phosphoribosylpyrophosphate amidotransferase	purF	4.15	6.05E-03
SMU.896	conserved hypothetical protein		4.06	6.12E-03
SMU.1496	galactose-6-phosphate isomerase	lacA	4	1.87E-03
SMU.196c	putative transfer protein		4	1.26E-04
SMU.1040c	putative oxidoreductase, short-chain dehydrogenase/reductase		3.99	2.26E-03
SMU.1001	putative DNA processing Smf protein	smf	3.95	9.81E-08
SMU.1299c	putative acetate kinase		3.93	2.91E-03
SMU.631	hypothetical protein		3.85	2.21E-02
SMU.31	hypothetical protein		3.84	8.09E-03
SMU.37	putative phosphoribosylaminoimidazolecarboxamide formyltransferse/IMP cyclohydrolase	purH	3.8	7.76E-03
SMU.200	hypothetical protein		3.8	1.59E-02
SMU.206	hypothetical protein		3.77	7.75E-03
SMU.440	hypothetical protein		3.76	6.86E-06
SMU.2035	conserved hypothetical protein; possible bacteriocin immunity protein		3.74	9.56E-03
SMU.1382	putative 3-isopropylmalate dehydratase, large subunit	leuC	3.69	2.27E-03
SMU.166	hypothetical protein		3.67	2.52E-03
SMU.963c	putative deacetylase		3.66	3.49E-08
SMU.36	conserved hypothetical protein		3.65	4.98E-03
SMU.83	heat shock protein DnaJ (HSP-40)	dnaJ	3.64	1.81E-06
SMU.38c	putative transcriptional regulator		3.63	2.66E-03
SMU.1237c	hypothetical protein		3.59	1.39E-03
SMU.35	putative phosphoribosylglycinamide formyltransferase (GART)	purN	3.57	1.31E-02
SMU.493	formate acetyltransferase (pyruvate formate-lyase 2)	pfl2	3.52	2.22E-04
SMU.1115	lactate dehydrogenase	idh	3.51	3.77E-03

Table 1. (Continued)

SMU.209c	hypothetical protein		3.49	2.57E-04
SMU.211c	hypothetical protein		3.44	3.46E-02
SMU.270	putative PTS system, membrane component; possible ribulose-monophosphate PTS pathway enzyme IIC	ulaA	3.41	4.37E-03
SMU.1279c	putative cell division protein (cell shape determining protein)	rodA	3.4	1.64E-05
SMU.202c	hypothetical protein		3.39	2.94E-04
SMU.1025	putative transcriptional regulator		3.28	1.00E-02
SMU.1889c	hypothetical protein		3.26	1.06E-02
SMU.29	putative phosphoribosylaminoimidazole-succinocarboxamide synthase SAICAR synthethase		3.25	1.87E-03
SMU.138	putative malate permease		3.23	2.18E-02
SMU.201c	putative transposon protein		3.23	6.70E-04
SMU.125	conserved hypothetical protein		3.21	5.04E-03
SMU.1657c	putative nitrogen regulatory protein PII		3.2	2.92E-03
SMU.141	conserved hypothetical protein		3.17	2.65E-02
SMU.199c	hypothetical protein		3.17	5.63E-03
SMU.81	heat shock protein GrpE (HSP-70 cofactor)	grpE	3.15	1.59E-03
SMU.843	hypothetical protein, poly-gamma-glutamate synthesis protein (capsule biosynthesis protein)		3.14	9.70E-04
SMU.1966c	putative periplasmic sugar-binding protein		3.11	1.13E-02
SMU.789	conserved hypothetical protein		3.09	7.96E-03
SMU.168	putative transcriptional regulator		3.09	3.33E-02
SMU.1692	pyruvate-formate lyase activating enzyme	pflA	3.08	1.02E-04
SMU.180	putative oxidoreductase; possible fumarate reductase		3.05	2.06E-05
SMU.198c	putative conjugative transposon protein		3.01	1.87E-02
SMU.207c	putative transposon protein		3	5.90E-06
SMU.193c	conserved hypothetical protein		2.96	9.65E-05
SMU.30	putative phosphoribosylformylglycinamidine synthase, (FGAM synthase)	purL	2.9	2.02E-03
SMU.1592	putative dipeptidase PepQ	pepQ	2.89	3.70E-04
SMU.116	tagatose 1,6-aldolase	lacD2	2.88	7.22E-03
SMU.208c	putative transposon protein; possible DNA segregation ATPase		2.87	2.60E-08
SMU.214c	hypothetical protein		2.87	1.05E-02
SMU.80	transcriptional regulator; repressor (HrcA) of class I heat shock genes	hrcA	2.85	6.66E-05
SMU.2061	hypothetical protein		2.85	3.22E-03
SMU.895	possible DNA-damage-inducible protein		2.84	1.68E-02
SMU.809	excinuclease ABC (subunit B); helicase subunit of the DNA excision repair complex	uvrB	2.83	4.21E-04
SMU.137	malate dehydrogenase	mleS	2.82	2.07E-02
SMU.58	hypothetical protein		2.79	2.15E-03
SMU.1681	conserved hypothetical protein		2.79	2.01E-02
SMU.458	putative ATP-dependent RNA helicase		2.78	2.55E-02
SMU.123	DNA polymerase III, alpha subunit	polC	2.78	6.19E-04
SMU.212c	hypothetical protein		2.77	7.61E-03
SMU.99	fructose-1,6-biphosphate aldolase	fbaA	2.76	9.03E-03
SMU.1325	putative ABC transporter, ATP-binding component	ftsE	2.76	3.45E-09
SMU.885	galactose operon repressor	galR	2.76	2.05E-03
SMU.82	heat shock protein, DnaK (HSP-70)	Dnak	2.74	1.40E-03
SMU.640c	putative transcriptional regulator (GntR family)		2.73	9.22E-03
SMU.1515	hypothetical protein	covX	2.72	5.29E-03
SMU.1120	putative sugar ABC transporter, ATP-binding protein		2.72	5.31E-06
SMU.167	hypothetical protein		2.72	1.29E-02

Table 1. (Continued)

SMU.139	conserved hypothetical protein		2.71	3.45E-04
SMU.765	NADH oxidase/alkyl hydroperoxidase reductase peroxide-forming		2.7	1.10E-02
SMU.2098	putative arginyl-tRNA synthase	argS	2.69	2.67E-02
SMU.1122	putative cytidine deaminase, cdd	cdd	2.68	5.36E-03
SMU.349	dimethyladenosine transferase		2.67	1.06E-02
SMU.491	putative DeoR-type transcriptional regulator		2.66	1.28E-03
SMU.691	putative tripeptidase (peptidase T)	pepT	2.63	2.19E-03
SMU.985	putative beta-glucosidase	bglA	2.62	1.27E-02
SMU.1659c	conserved hypothetical protein,		2.62	2.95E-03
SMU.428	conserved hypothetical protein		2.6	3.74E-03
SMU.1029	conserved hypothetical protein		2.58	1.47E-03
SMU.1664c	putative acetoin utilization protein, acetoin dehydrogenase	acuB	2.56	3.13E-02
SMU.194c	conserved hypothetical protein; Bacteriophage P2 associated		2.55	1.08E-03
SMU.764	alkyl hydroperoxide reductase	ahpC	2.55	1.48E-02
SMU.2096c	hypothetical protein		2.52	2.35E-02
SMU.1153c	hypothetical protein		2.5	8.95E-03
SMU.1039c	putative lipopolysaccharide glycosyltransferase		2.49	1.49E-03
SMU.1683c	conserved hypothetical protein		2.48	9.24E-07
SMU.402	pyruvate formate-lyase	pflA	2.48	1.49E-02
SMU.1014	hypothetical protein		2.47	1.02E-02
SMU.205c	hypothetical protein		2.47	1.08E-06
SMU.1474c	conserved hypothetical protein-ribonuclease Z		2.46	1.00E-02
SMU.213c	hypothetical protein		2.44	1.27E-02
SMU.495	glycerol dehydrogenase	gldA	2.43	1.10E-02
SMU.1016	putative acetyl-CoA carboxylase, biotin carboxyl carrier subunit	bcc	2.41	2.72E-02
SMU.1324	putative cell-division protein FtsX	ftsX	2.41	2.41E-02
SMU.309	regulator of sorbitol operon		2.4	5.81E-06
SMU.191c	putative integrase		2.4	2.94E-04
SMU.743	conserved hypothetical protein		2.37	4.05E-03
SMU.878	multiple sugar-binding ABC transporter, sugar-binding protein presursor MsmE	msmE	2.37	3.42E-03
SMU.1121c	putative ABC transporter		2.36	3.94E-03
SMU.984	hypothetical protein		2.36	5.78E-03
SMU.489	putative polyribonucleotide nucleotidyltransferase (general stress protein 13)		2.35	6.83E-04
SMU.1035	putative ABC transporter, ATP-binding protein	glrA	2.34	2.11E-02
SMU.1106c	conserved hypothetical protein; phosphoglycerate mutase-like protein		2.34	4.12E-03
SMU.1405c	conserved hypothetical protein		2.32	7.21E-03
SMU.1471c	conserved hypothetical protein		2.32	3.75E-03
SMU.262	putative ornithine carbamoyltransferase	otcA	2.32	1.57E-04
SMU.1287	putative transcriptional regulator		2.29	2.57E-08
SMU.305	hypothetical protein		2.25	6.23E-03
SMU.46	hypothetical protein		2.25	8.93E-03
SMU.1409c	putative transcriptional regulator		2.25	1.95E-02
SMU.364	glutamine synthetase type 1; glutamate--ammonia ligase	glnA	2.24	1.86E-02
SMU.274	putative L-xylulose 5-phosphate 3-epimerase		2.24	9.77E-04
SMU.140	putative glutathione reductase		2.24	5.19E-04
SMU.1687	putative manganese-dependent inorganic pyrophosphatase	ppaC	2.23	1.61E-04
SMU.1579	hypothetical protein		2.23	9.83E-03
SMU.115	putative PTS system, fructose-specific IIA component		2.22	9.36E-06
SMU.395	X-prolyl dipeptidyl peptidase	pepX	2.22	3.65E-02

Table 1. (Continued)

SMU.2113c	conserved hypothetical protein		2.22	1.28E-02
SMU.204c	hypothetical protein		2.2	1.78E-03
SMU.318	putative hippurate hydrolase		2.19	4.57E-03
SMU.431	putative ABC transporter, ATP-binding protein		2.19	3.96E-03
SMU.664	putative ornithine acetyltransferase/N-acetylglutamate synthase	argJ	2.18	3.09E-03
SMU.1598	cellobiose phosphotransferase system IIA component	celC	2.18	2.39E-02
SMU.1650	putative endonuclease III (DNA repair)		2.14	4.99E-03
SMU.1804c	hypothetical protein		2.14	3.39E-02
SMU.1119c	putative sugar ABC transporter, permease protein		2.14	5.63E-04
SMU.404c	hypothetical protein		2.14	2.57E-07
SMU.837	putative reductase		2.14	1.40E-02
SMU.1406c	conserved hypothetical protein		2.13	1.65E-02
SMU.210c	hypothetical protein		2.13	2.11E-07
SMU.133c	putative MDR permease; transmembrane efflux protein		2.12	2.89E-02
SMU.1177c	putative ABC transporter, glutamine binding protein		2.11	2.60E-09
SMU.45	hypothetical protein		2.11	1.26E-02
SMU.290	putative L-ascorbate 6-phosphate lactonase		2.1	2.65E-02
SMU.43	putative site-specific DNA-methyltransferase restriction-modification protein		2.1	3.24E-04
SMU.197c	hypothetical protein		2.09	3.08E-04
SMU.1484c	conserved hypothetical protein		2.08	6.90E-05
SMU.1601	6- phospho-beta-glucosidase	celA	2.08	8.31E-03
SMU.1167c	putative ABC transporter, ATP-binding protein		2.07	2.56E-02
SMU.1679c	conserved hypothetical protein		2.07	6.33E-03
SMU.2056	putative ATPase, recombination factor protein RarA	rarA	2.06	7.68E-06
SMU.1291c	putative chorismate mutase		2.06	8.47E-04
SMU.897	putative type I restriction-modification system, helicase subunits		2.06	2.12E-02
SMU.817	putative amino acid transporter, amino acid-binding protein		2.06	1.23E-02
SMU.273	putative hexulose-6-phosphate synthase 3-keto-L-gulonate-6-phosphate decarboxylase	ulaD	2.06	6.61E-03
SMU.1589c	putative hexosyltransferase		2.05	5.41E-03
SMU.1107c	conserved hypothetical protein		2.05	1.86E-02
SMU.844	conserved hypothetical protein		2.05	6.15E-06
SMU.1627	50S ribosomal L11 protein	rplK	2.05	5.44E-03
SMU.813	hypothetical protein; putative transcriptional regulator		2.04	3.89E-02
SMU.1839	mannose-6-phosphate isomerase	manA	2.04	2.39E-02
SMU.1954	putative chaperonin GroEL	groEL	2.04	4.00E-03
SMU.886	galactokinase	galK	2.04	2.95E-02
SMU.216c	hypothetical protein		2.03	4.69E-02
SMU.1581	DNA polymerase III, gamma/tau subunit	dnaX	2.02	1.69E-02
SMU.399	conserved hypothetical protein		2.01	7.01E-03
SMU.876	putative MSM operon regulatory protein	msmR	2	3.68E-02
SMU.1221	putative orotate phosphoribosyltransferase	pyrE	2	2.63E-03
	down-regulated genes			
SMU.496	putative cysteine synthetase A; O-acetylserine lyase	cysK	0.13	5.50E-03
SMU.1882c	hypothetical protein		0.15	1.19E-03
SMU.1340	putative surfactin synthetase	bacA2	0.17	4.02E-03
SMU.1182	mannitol-1-phosphate dehydrogenase	mtlD	0.19	5.42E-05
SMU.1339	putative bacitracin synthetase	bacD	0.19	2.77E-03
SMU.1061	putative DNA-binding protein	ylxM	0.2	7.98E-04
SMU.618	hypothetical protein		0.21	2.18E-03

Table 1. (Continued)

SMU.2001	DNA-directed RNA polymerase, alpha subunit	rpoA	0.21	6.03E-03
SMU.2004	putative translation initiation factor IF-1	infA	0.21	2.07E-03
SMU.1338c	putative permease; possible multidrug-efflux transporter		0.22	2.36E-03
SMU.1336	conserved hypothetical protein PksD, involved in polyketide synthesis	pksD	0.22	6.37E-04
SMU.933	putative amino acid ABC transporter, periplasmic amino acid-binding protein		0.22	2.11E-03
SMU.2002	30S ribosomal protein S11	rs11	0.22	1.37E-02
SMU.1341c	putative gramicidin S synthetase		0.23	1.27E-02
SMU.616	hypothetical protein		0.23	3.70E-05
SMU.1236c	conserved hypothetical protein		0.24	8.19E-03
SMU.2003	30S ribosomal protein S13	rpsM	0.24	2.01E-03
SMU.2028	levansucrase precursor; beta-D-fructosyltransferase	sacB/ftf	0.24	3.28E-03
SMU.1762c	conserved hypothetical protein		0.25	2.09E-02
SMU.934	putative amino acid ABC transporter, permease protein		0.25	9.32E-04
SMU.932	hypothetical protein		0.25	7.51E-04
SMU.1622	putative peptide methionine sulfoxide reductase	pmsR	0.25	3.04E-04
SMU.1342	putative bacitracin synthetase 1	bacA1	0.26	1.73E-02
SMU.1513	putative chromosome segregation ATPase; SMC protein	smc	0.26	1.84E-03
SMU.2003c	50S ribosomal protein L36_large subunit	rpmJ	0.26	5.87E-03
SMU.277	hypothetical protein		0.26	1.14E-02
SMU.2005	putative adenylate kinase	adk	0.28	3.42E-03
SMU.949	ATP-dependent protease Clp, ATPase subunit	clpX	0.28	1.63E-03
SMU.1532	FoF1 membrane-bound proton-translocating ATPase, b subunit	atpF	0.28	3.79E-03
SMU.1552c	hypothetical protein		0.29	1.65E-03
SMU.1335c	putative enoyl-(acyl-carrier-protein) reductase		0.29	4.47E-03
SMU.961	conserved hypothetical protein		0.29	2.13E-03
SMU.1530	FoF1 membrane-bound proton-translocating ATPase, alpha subunit	atpD	0.29	3.21E-03
SMU.1764c	conserved hypothetical protein		0.3	3.58E-02
SMU.1646c	conserved hypothetical protein, possible hemolysis inducing protein		0.3	1.16E-03
SMU.776	conserved hypothetical protein		0.3	4.42E-04
SMU.722	hypothetical protein		0.3	2.05E-06
SMU.1511c	putative acetyltransferase		0.31	1.71E-03
SMU.339	hypothetical protein		0.31	6.72E-09
SMU.2007	50S ribosomal protein L15	rplO	0.31	2.55E-02
SMU.1343c	putative polyketide synthase		0.32	2.45E-02
SMU.1528	FoF1 membrane-bound proton-translocating ATPase, beta subunit	atpB	0.32	9.95E-03
SMU.948	conserved hypothetical protein		0.32	2.58E-04
SMU.285	hypothetical protein		0.32	2.52E-02
SMU.1188	putative signal peptidase	lepB	0.33	1.17E-04
SMU.1743	putative acyl carrier protein	acpP	0.33	3.30E-02
SMU.1545c	conserved hypothetical protein		0.33	3.89E-03
SMU.278	hypothetical protein		0.33	3.96E-03
SMU.1770	putative valyl-tRNA synthetase	valS	0.33	1.05E-03
SMU.2012	30S ribosomal protein S8	rpsH	0.33	3.10E-02
SMU.777	putative 3-dehydroquinate dehydratase	aroD	0.34	9.50E-04
SMU.2014	30S ribosomal protein S14	rpsN	0.34	8.56E-03
SMU.1345c	putative peptide synthetase similar to MycA		0.34	8.79E-05
SMU.988	putative cardiolipin synthase		0.34	9.11E-03
SMU.453	conserved hypoyhetical protein; S-adenosyl-methyltransferase	mraW	0.34	7.55E-03
SMU.1081	conserved hypothetical protein		0.34	9.26E-03
SMU.258	putative oligopeptide ABC transporter, ATP-binding protein	oppD	0.34	2.18E-02

Table 1. (Continued)

SMU.1902c	hypothetical protein		0.35	7.97E-03
SMU.1941	putative membrane lipoprotein	atmB	0.35	1.84E-03
SMU.1736	putative acetyl-CoA carboxylase biotin carboxylase subunit	accC	0.35	8.16E-03
SMU.1330c	putative transposase		0.35	7.42E-03
SMU.1246c	putative transcriptional regulator		0.35	3.29E-02
SMU.1512	putative phenylalanyl-tRNA synthetase (alpha subunit)	pheS	0.36	5.34E-03
SMU.830	RgpFc protein; polysaccharide biosynthesis protein		0.36	2.38E-02
SMU.336	putative ribonuclease P protein component	rnpA	0.36	4.81E-05
SMU.2026c	30S ribosomal protein S10		0.36	7.46E-04
SMU.1881c	putative ABC transporter, ATP-binding protein		0.37	4.56E-05
SMU.1913c	putative immunity protein, BLpL-like		0.37	1.72E-02
SMU.620	hypothetical protein		0.37	3.78E-03
SMU.1334	putative phosphopantetheinyl transferase	sfp	0.38	3.66E-03
SMU.1869	putative thioredoxin	trxA	0.38	3.79E-02
SMU.1082	putative serine hydroxymethyltransferase	glyA	0.38	3.48E-04
SMU.1080c	conserved hypothetical protein; possible transposon-related protein		0.39	1.83E-02
SMU.1912c	hypothetical protein		0.39	8.11E-03
SMU.557	putative cell division protein	divIVA	0.39	1.83E-02
SMU.697	putative translation initiation factor IF3	infC	0.39	3.68E-04
SMU.989	aspartate-semialdehyde dehydrogenase, asd	asd	0.4	1.02E-07
SMU.1191	6-phosphofructokinase	pfkA	0.4	8.65E-03
SMU.1876	conserved hypothetical protein		0.4	2.35E-02
SMU.936	putative amino acid ABC transporter, ATP-binding protein		0.4	1.86E-02
SMU.546	putative GTP-binding protein		0.41	6.31E-04
SMU.713	putative cell division protein	ftsW	0.41	9.37E-04
SMU.1396	glucan-binding protein C	gbpC	0.41	7.23E-04
SMU.1656	putative phosphoserine aminotransferase	serC	0.41	1.32E-02
SMU.460	putative amino acid ABC transporter, permease		0.41	2.23E-03
SMU.1939c	putative ABC transporter, ATP-binding protein		0.41	5.98E-04
SMU.1910c	hypothetical protein		0.41	1.73E-02
SMU.2025c	50S ribosomal protein L3	rplC	0.41	1.10E-02
SMU.155	polyribonucleotide nucleotidyltransferase	pnpA	0.41	9.90E-05
SMU.1923c	conserved hypothetical protein		0.41	1.64E-03
SMU.1079c	putative ABC transporter, ATP-binding protein		0.41	3.87E-02
SMU.1381	putative 3-isopropylmalate dehydratase, small subunit	leuD	0.42	1.34E-02
SMU.1063	putative ABC transporter, ATP-binding protein, proline/glycine betaine transport system	opuAa	0.42	4.15E-02
SMU.2015	50S ribosomal protein L5	rplE	0.42	3.27E-02
SMU.1909c	hypothetical protein		0.42	1.40E-02
SMU.1331c	putative transposase		0.42	6.42E-03
SMU.1940c	hypothetical protein		0.42	7.70E-03
SMU.1098c	putative oxidoreductase		0.42	7.90E-03
SMU.952	putative methyltransferase; homocysteine methyltransferase	mmuM	0.43	8.09E-04
SMU.555	conserved hypothetical protein	ylmG	0.43	6.35E-03
SMU.2000	50S ribosomal protein L17	rplQ	0.43	2.03E-02
SMU.156	conserved hypothetical protein		0.43	5.97E-05
SMU.556	conserved hypothetical protein	ylmH	0.43	1.43E-02
SMU.1189c	conserved hypothetical protein		0.43	2.01E-02
SMU.1570	putative maltose/maltodextrin ABC transporter, MalG permease	malG	0.43	1.13E-02
SMU.409	conserved hypothetical protein		0.43	1.65E-04
SMU.944	thymidylate synthase	thyA	0.43	1.62E-04

Table 1. (Continued)

SMU.2001	DNA-directed RNA polymerase, alpha subunit	rpoA	0.21	6.03E-03
SMU.2004	putative translation initiation factor IF-1	infA	0.21	2.07E-03
SMU.1338c	putative permease; possible multidrug-efflux transporter		0.22	2.36E-03
SMU.1336	conserved hypothetical protein PksD, involved in polyketide synthesis	pksD	0.22	6.37E-04
SMU.933	putative amino acid ABC transporter, periplasmic amino acid-binding protein		0.22	2.11E-03
SMU.2002	30S ribosomal protein S11	rs11	0.22	1.37E-02
SMU.1341c	putative gramicidin S synthetase		0.23	1.27E-02
SMU.616	hypothetical protein		0.23	3.70E-05
SMU.1236c	conserved hypothetical protein		0.24	8.19E-03
SMU.2003	30S ribosomal protein S13	rpsM	0.24	2.01E-03
SMU.2028	levansucrase precursor; beta-D-fructosyltransferase	ftf	0.24	3.28E-03
SMU.1762c	conserved hypothetical protein		0.25	2.09E-02
SMU.934	putative amino acid ABC transporter, permease protein		0.25	9.32E-04
SMU.932	hypothetical protein		0.25	7.51E-04
SMU.1622	putative peptide methionine sulfoxide reductase	pmsR	0.25	3.04E-04
SMU.1342	putative bacitracin synthetase 1	bacA1	0.26	1.73E-02
SMU.1513	putative chromosome segregation ATPase; SMC protein	smc	0.26	1.84E-03
SMU.2003c	50S ribosomal protein L36_large subunit	rpmJ	0.26	5.87E-03
SMU.277	hypothetical protein		0.26	1.14E-02
SMU.2005	putative adenylate kinase	adk	0.28	3.42E-03
SMU.949	ATP-dependent protease Clp, ATPase subunit	clpX	0.28	1.63E-03
SMU.1532	FoF1 membrane-bound proton-translocating ATPase, b subunit	atpF	0.28	3.79E-03
SMU.1552c	hypothetical protein		0.29	1.65E-03
SMU.1335c	putative enoyl-(acyl-carrier-protein) reductase		0.29	4.47E-03
SMU.961	conserved hypothetical protein		0.29	2.13E-03
SMU.1530	FoF1 membrane-bound proton-translocating ATPase, alpha subunit	atpD	0.29	3.21E-03
SMU.1764c	conserved hypothetical protein		0.3	3.58E-02
SMU.1646c	conserved hypothetical protein, possible hemolysis inducing protein		0.3	1.16E-03
SMU.776	conserved hypothetical protein		0.3	4.42E-04
SMU.722	hypothetical protein		0.3	2.05E-06
SMU.1511c	putative acetyltransferase		0.31	1.71E-03
SMU.339	hypothetical protein		0.31	6.72E-09
SMU.2007	50S ribosomal protein L15	rplO	0.31	2.55E-02
SMU.1343c	putative polyketide synthase		0.32	2.45E-02
SMU.1528	FoF1 membrane-bound proton-translocating ATPase, beta subunit	atpB	0.32	9.95E-03
SMU.948	conserved hypothetical protein		0.32	2.58E-04
SMU.285	hypothetical protein		0.32	2.52E-02
SMU.1188	putative signal peptidase	lepB	0.33	1.17E-04
SMU.1743	putative acyl carrier protein	acpP	0.33	3.30E-02
SMU.1545c	conserved hypothetical protein		0.33	3.89E-03
SMU.278	hypothetical protein		0.33	3.96E-03
SMU.1770	putative valyl-tRNA synthetase	valS	0.33	1.05E-03
SMU.2012	30S ribosomal protein S8	rpsH	0.33	3.10E-02
SMU.777	putative 3-dehydroquinate dehydratase	aroD	0.34	9.50E-04
SMU.2014	30S ribosomal protein S14	rpsN	0.34	8.56E-03
SMU.1345c	putative peptide synthetase similar to MycA		0.34	8.79E-05
SMU.988	putative cardiolipin synthase		0.34	9.11E-03
SMU.453	conserved hypoyhetical protein; S-adenosyl-methyltransferase	mraW	0.34	7.55E-03
SMU.1081	conserved hypothetical protein		0.34	9.26E-03
SMU.258	putative oligopeptide ABC transporter, ATP-binding protein	oppD	0.34	2.18E-02

Table 1. (Continued)

SMU.2137c	conserved hypothetical protein		0.49	4.40E-02
SMU.66	conserved hypothetical protein		0.49	4.27E-02
SMU.1332c	putative transposase		0.49	4.39E-03
SMU.256	putative oligopeptide transport system, permease protein	oppB	0.49	8.57E-03
SMU.2154c	putative peptidase		0.49	3.36E-03
SMU.942	putative hydroxymethylglutaryl-CoA reductase	mvaA	0.5	7.98E-03
SMU.971	putative 2-amino-4-hydroxy-6-hydroxymethylpteridine pyrophosphokinas	folK	0.5	1.19E-02
SMU.340	50S ribosomal protein L34	rpmH	0.5	1.45E-09
SMU.464	putative nicotinate phosphoribosyltransferase		0.5	8.07E-06
SMU.1738	putative biotin carboxyl carrier protein of acetyl-CoA carboxylase	bccP	0.5	1.13E-02

Table 2. Differentially expressed genes in biofilms supplemented with glucose *vs.* biofilms supplemented with sucrose in stationary phase (p-value<0.05). Ratio represents the value for each differentially expressed gene in biofilms supplemented with sucrose.

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.10	hypothetical protein		6.51	2.83E-02
SMU.840c	hypothetical protein		6.18	2.11E-03
SMU.1387	putative oxidoreductase		6.07	2.69E-02
SMU.09	hypothetical protein		5.9	2.22E-03
SMU.2155	hypothetical protein		5.71	1.30E-03
SMU.1052	hypothetical protein		5.51	1.57E-02
SMU.768c	hypothetical protein		5.36	9.86E-03
SMU.218	putative transcriptional regulator		5.36	1.40E-02
SMU.504	putative site-specific DNA-methyltransferase	dam	5.2	2.43E-02
SMU.510c	hypothetical protein		5.11	6.87E-03
SMU.1956c	hypothetical protein		5.03	3.50E-02
SMU.1360c	hypothetical protein		4.92	3.37E-02
SMU.12	hypothetical protein		4.78	4.90E-02
SMU.1773c	hypothetical protein		4.61	3.14E-02
SMU.44	DNA mismatch repair protein		4.45	3.98E-02
SMU.1576c	hypothetical protein		4.45	2.49E-02
SMU.1781	hypothetical protein		4.31	1.01E-02
SMU.1161c	hypothetical protein		4.26	3.12E-02
SMU.1771c	hypothetical protein		4.26	4.05E-02
SMU.05	hypothetical protein		4.06	1.18E-02
SMU.46	hypothetical protein		4.06	3.74E-02
SMU.1034c	site-specific tyrosine recombinase	xerS	3.96	9.47E-03
SMU.47	hypothetical protein		3.95	3.35E-02
SMU.1753c	hypothetical protein		3.93	2.36E-02
SMU.11	hypothetical protein		3.93	4.96E-02
SMU.1095	putative choline ABC transporter, osmoprotectant binding protein	opuBc	3.84	4.76E-03
SMU.1577c	hypothetical protein		3.83	2.29E-02
SMU.2156	putative RecF protein, ATPase involved in DNA repair	recF	3.74	4.68E-02
SMU.1349	hypothetical protein		3.73	2.43E-02
SMU.1158c	hypothetical protein		3.7	3.10E-02
SMU.43	putative site-specific DNA-methyltransferase restriction-modification protein		3.67	3.72E-02
SMU.505	putative adenine-specific DNA methylase		3.61	3.79E-02
SMU.1798c	hypothetical protein		3.59	4.18E-02
SMU.1460	putative dTDP-4-keto-L-rhamnose reductase	rmlC	3.52	4.10E-02
SMU.2137	hypothetical protein		3.52	1.87E-02
SMU.526c	putative transcriptional regulator		3.5	2.79E-02
SMU.2084c	transcriptional regulator Spx	spxA	3.33	7.66E-03
SMU.506	putative type II restriction endonuclease		3.29	4.02E-02
SMU.1728	transcription elongation factor	greA	3.18	1.22E-02
SMU.1922	putative chromosome replication protein	dnaB	3.04	2.30E-02
SMU.2061	hypothetical protein		3.04	3.99E-02
SMU.1688	putative extramembranal protein	dltD	3	4.60E-02
SMU.02	putative DNA polymerase III, beta subunit	dnaN	2.99	3.22E-02
SMU.1340	putative surfactin synthetase	bacA2	2.96	1.79E-02
SMU.986c	hypothetical protein		2.94	3.76E-02

Table 2. (Continued)

SMU.2164	serine protease HtrA	htrA	2.88	4.77E-02
SMU.1488c	hypothetical protein		2.72	1.03E-02
SMU.01	chromosomal replication initiator protein	dnaA	2.7	1.24E-02
SMU.1126	putative pantothenate kinase	coaA	2.67	4.45E-02
SMU.1671c	hypothetical protein		2.66	2.35E-02
SMU.1941	putative membrane lipoprotein	atmB	2.65	5.23E-03
SMU.1903c	hypothetical protein		2.64	6.68E-04
SMU.1409c	putative transcriptional regulator		2.63	2.19E-02
SMU.682	hypothetical protein		2.57	1.87E-02
SMU.2154c	putative peptidase		2.52	1.81E-02
SMU.700c	putative phosphoglycerate mutase-like protein		2.48	1.84E-02
SMU.1902c	hypothetical protein		2.47	5.50E-03
SMU.2134	putative transcriptional regulator		2.44	2.62E-02
SMU.1343c	putative polyketide synthase		2.42	2.63E-04
SMU.2104a	50S ribosomal protein L32	rpmF	2.37	2.67E-04
SMU.1904c	hypothetical protein		2.32	2.37E-05
SMU.999	hypothetical protein		2.32	2.87E-02
SMU.1713c	segregation and condensation protein A	scpA	2.29	3.26E-02
SMU.1358	putative transposase fragment		2.27	3.62E-02
SMU.1129	putative response regulator	ciaR	2.26	2.23E-02
SMU.394c	onserved hypothetical protein		2.22	2.87E-02
SMU.1342	putative bacitracin synthetase 1	bacA1	2.19	1.00E-02
SMU.2153c	putative peptidase		2.14	1.81E-02
SMU.1900	hypothetical protein		2.14	1.90E-02
SMU.1339	putative bacitracin synthetase	bscD	2.14	1.75E-02
SMU.1336	hypothetical protein PksD, involved in polyketide synthesis	pksD	2.12	2.02E-02
SMU.369c	hypothetical protein		2.11	2.73E-02
SMU.1182	mannitol-1-phosphate 5-dehydrogenase	mtlD	2.11	1.48E-04
SMU.669c	putative glutaredoxin		2.09	4.18E-02
SMU.2106c	transcriptional regulator		2.07	3.74E-02
SMU.2093	putative transcriptional regulator of arginine metabolism	argR	2.03	4.66E-02
SMU.105	putative transcriptional regulator; repressor of sugar transport operon		2.03	2.31E-02
SMU.37	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	purH	2.02	1.11E-02
	down-regulated genes			
SMU.575c	holin-like protein LrgA	lrgA	0.05	9.11E-03
SMU.574c	holin-like protein LrgB	lrgB	0.17	2.56E-02
SMU.764	alkyl hydroperoxide reductase	ahpC	0.25	2.06E-02
SMU.424	negative transcriptional regulator	copY	0.25	3.64E-02
SMU.2127	putative succinate semialdehyde dehydrogenase		0.27	2.37E-07
SMU.179	hypothetical protein		0.29	1.39E-02
SMU.670	aconitate hydratase; aconitase	citB	0.29	1.71E-02
SMU.1674	putative aminotransferase; probable beta-cystathionase	metC	0.3	3.65E-02
SMU.631	hypothetical protein		0.32	7.82E-03
SMU.857	putative uracil permease		0.33	4.41E-02
SMU.127	putative acetoin dehydrogenase (TPP-dependent) E1 component alpha subunit	adhA	0.33	7.13E-03
SMU.247	putative ABC transporter, ATP-binding protein		0.33	2.36E-02
SMU.1273	putative histidinol-phosphate aminotransferase	hisC	0.34	1.30E-03
SMU.252	hypothetical protein		0.35	1.29E-05

Table 2. (Continued)

SMU.957	50S ribosomal protein L10	rplJ	0.36	4.50E-02
SMU.1077	putative phosphoglucomutase	pgm	0.37	4.91E-02
SMU.858	putative aspartate transcarbamoylase	pyrB	0.37	1.68E-02
SMU.859	putative carbamoyl phosphate synthetase, small subunit	pyrA	0.37	1.33E-02
SMU.1016	putative acetyl-CoA carboxylase, biotin carboxyl carrier subunit	bcc	0.37	5.34E-03
SMU.965	homoserine dehydrogenase		0.38	3.39E-02
SMU.672	isocitrate dehydrogenase	idh	0.38	4.01E-02
SMU.765	NADH oxidase/alkyl hydroperoxidase reductase peroxide-forming		0.38	1.24E-04
SMU.1611c	putative permease; possible multi-drug resistance efflux pump		0.39	4.79E-03
SMU.674	phosphoenolpyruvate:sugar phosphotransferase system HPr	pstH	0.39	3.17E-02
SMU.361	phosphoglycerate kinase	pgk	0.39	1.23E-03
SMU.1586	putative threonyl-tRNA synthetase	thrS	0.39	7.46E-03
SMU.2074	putative anaerobic ribonucleoside-triphosphate reductase	nrdD	0.39	4.07E-07
SMU.879	multiple sugar-binding ABC transporter, permease protein	msmF	0.39	1.56E-02
SMU.1031	putative transposon excisionase; Tn916 ORF1-like	xis	0.39	9.30E-03
SMU.92c	hypothetical protein; putative transposase fragment		0.4	1.33E-02
SMU.1539	putative 1,4-alpha-glucan branching enzyme	glgB	0.4	1.93E-02
SMU.1520	putative ABC transporter, glutamine binding protein		0.4	4.95E-02
SMU.880	multiple sugar-binding ABC transporter, permease protein	msmG	0.41	3.18E-02
SMU.128	putative acetoin dehydrogenase (TPP-dependent), E1 component beta subunit	adhB	0.41	1.78E-03
SMU.365	glutamate synthase (large subunit)	gltA	0.41	4.84E-05
SMU.2101	aspartyl-tRNA synthetase	aspS	0.41	1.56E-02
SMU.442	hypothetical protein		0.41	1.47E-02
SMU.1223	dihydroorotate dehydrogenase B	pyrDB	0.42	6.61E-04
SMU.129	putative dihydrolipoamide acetyltransferase	adhC	0.42	3.59E-02
SMU.286	putative ABC transporter, ATP-binding protein	comA	0.42	2.59E-02
SMU.671	citrate synthase, citZ	citZ	0.42	3.64E-02
SMU.977	putative transcriptional antiterminator LicT (fragment)	licT	0.42	3.33E-02
SMU.500	putative ribosome-associated protein		0.42	6.24E-03
SMU.673	hypothetical protein		0.42	1.05E-03
SMU.1043c	putative phosphotransacetylase	eutD	0.42	1.16E-02
SMU.1569	putative maltose/maltodextrin ABC transporter, permease protein	malF	0.43	1.28E-02
SMU.441	putative transcriptional regulator		0.43	2.45E-02
SMU.1590	intracellular alpha-amylase	amyA	0.43	2.01E-02
SMU.440	hypothetical protein		0.43	6.82E-04
SMU.270	ascorbate-specfic PTS system enzyme II C	ulaA	0.43	2.83E-02
SMU.796	hypothetical protein		0.44	1.15E-02
SMU.629	putative manganese-type superoxide dismutase, Fe/Mn-SOD	sod	0.44	1.62E-02
SMU.115	putative PTS system, fructose-specific IIA component		0.44	3.36E-03
SMU.877	alpha-galactosidase	agaL	0.45	1.02E-02
SMU.1177c	putative ABC transporter, glutamine binding protein		0.46	2.81E-02
SMU.2047	putative PTS system, glucose-specific IIABC component	ptsG	0.47	3.17E-02
SMU.1001	putative DNA processing Smf protein	smf	0.47	1.14E-02
SMU.1014	hypothetical protein		0.47	2.05E-02
SMU.794	hypothetical protein		0.48	4.08E-02
SMU.273	3-keto-L-gulonate-6-phosphate decarboxylase	ulaD	0.49	4.59E-02
SMU.1220c	hypothetical protein		0.49	8.53E-06
SMU.1120	putative sugar ABC transporter, ATP-binding protein		0.49	6.15E-04
SMU.249	putative NifS protein homologue, class-V aminotransferase	nifS	0.5	1.85E-03
SMU.795	hypothetical protein; probable esterase		0.5	1.30E-02

Table 3. Differentially expressed genes in "starved" biofilms *vs* "fed" biofilms (p-value<0.05). Ratio represents the value for each differentially expressed gene in "fed biofilms".

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.1168	putative transcriptional regulator		14.7	8.36E-09
SMU.2001	DNA-directed RNA polymerase, alpha subunit	rpoA	9.73	1.51E-07
SMU.2002	30S ribosomal protein S11	rs11	7.05	3.13E-05
SMU.2004	putative translation initiation factor IF-1	infA	6.88	1.30E-07
SMU.957	50S ribosomal protein L10	rplJ	6.56	1.02E-02
SMU.753	hypothetical protein		6.08	6.42E-03
SMU.1288	50S ribosomal protein L19	rplS	5.94	1.68E-05
SMU.1047c	hypothetical protein		5.84	8.69E-08
SMU.1061	putative DNA-binding protein	ylxM	5.77	6.86E-05
SMU.1902c	hypothetical protein		5.66	3.09E-03
SMU.2032	30S ribosomal protein S2	rpsB	5.65	4.18E-03
SMU.339	hypothetical protein		5.49	1.73E-03
SMU.2000	50S ribosomal protein L17	rplQ	5.47	3.64E-04
SMU.758c	hypothetical protein		5.46	1.49E-02
SMU.358	30S ribosomal protein S7		5.39	5.11E-03
SMU.357	30S ribosomal protein S12	rpsL	5.39	1.19E-07
SMU.865	30S ribosomal protein S16	rpsP	5.36	9.71E-05
SMU.340	50S ribosomal protein L34	rpmH	5.29	3.78E-02
SMU.968	GTP cyclohydrolase I	folE	4.89	2.68E-02
SMU.589	putative DNA-binding protein		4.84	4.73E-06
SMU.1841	putative PTS system, sucrose-specific IIABC component	scrA	4.71	2.21E-05
SMU.2037	putative trehalose-6-phosphate hydrolase	treA	4.69	3.70E-02
SMU.1745c	putative transcriptional regulator		4.68	3.67E-07
SMU.154	30S ribosomal protein S15	rpsO	4.68	7.33E-03
SMU.1703c	hypothetical protein		4.57	3.94E-03
SMU.1646c	hemolysis inducing protein		4.49	1.56E-02
SMU.1924	response regulator GcrR for glucan-binding protein C	gcrR	4.48	3.08E-07
SMU.1513	putative chromosome segregation ATPase; SMC protein	smc	4.48	6.85E-07
SMU.958	hypothetical protein		4.46	4.48E-06
SMU.846	50S ribosomal protein L21	rplU	4.42	9.34E-03
SMU.1502c	hypothetical protein		4.4	9.45E-04
SMU.1294	flavodoxin	flaW	4.4	1.32E-02
SMU.369c	hypothetical protein		4.31	6.47E-03
SMU.423	hypothetical protein		4.26	3.01E-03
SMU.634	S-adenosylmethionine--tRNA ribosyltransferase-isomerase	queA	4.21	1.29E-06
SMU.2003	30S ribosomal protein S13	rpsM	4.05	2.73E-02
SMU.604	hypothetical protein		4.03	3.83E-06
SMU.2083c	hypothetical protein		3.99	6.30E-03
SMU.1498	lactose repressor	lacR	3.98	9.18E-03
SMU.818	30S ribosomal protein S21	rpsU	3.98	9.73E-03
SMU.1859	single-stranded DNA-binding protein	ssb	3.97	2.05E-02
SMU.697	translation initiation factor IF3	infC	3.94	2.81E-02
SMU.2084c	transcriptional regulator Spx	spxA	3.94	1.14E-07
SMU.1882c	hypothetical protein		3.91	4.39E-07
SMU.564	hypothetical protein		3.9	1.29E-02
SMU.1249c	hypothetical protein		3.85	2.93E-06
SMU.1914c	hypothetical protein	bsmA	3.84	2.97E-02

Table 3. (Continued)

SMU.246	putative glycosyl transferase N-acetylglucosaminyltransferase	rgpG	3.82	9.25E-05
SMU.1200	30S ribosomal protein S1	rpsA	3.76	6.58E-03
SMU.1846c	hypothetical protein		3.75	1.06E-04
SMU.2104a	50S ribosomal protein L32	rpmF	3.69	4.59E-03
SMU.409	hypothetical protein		3.69	1.05E-02
SMU.1527	FoF1 membrane-bound proton-translocating ATPase, epsilon subunit	atpC	3.66	1.22E-06
SMU.1845	transcription antitermination protein NusB	nusB	3.65	1.97E-06
SMU.462	hypothetical protein		3.6	1.15E-03
SMU.1519	putative amino acid ABC transporter, ATP-binding protein	glnQ	3.58	1.49E-02
SMU.2003a	50S ribosomal protein L36	rpmJ	3.57	1.50E-04
SMU.453	S-adenosyl-methyltransferase	mraW	3.57	2.31E-06
SMU.1765c	hypothetical protein		3.56	6.49E-03
SMU.245	putative negative regulator of genetic competence	mecA	3.53	1.31E-04
SMU.1287	putative transcriptional regulator		3.51	2.42E-03
SMU.1931	glucose-inhibited division protein	gidB	3.46	2.61E-02
SMU.1179c	putative amino acid ABC transporter, permease protein		3.44	3.66E-02
SMU.2102	histidyl-tRNA synthetase	hisS	3.39	2.95E-02
SMU.747c	putative permease		3.35	2.31E-02
SMU.674	phosphoenolpyruvate:sugar phosphotransferase system HPr	ptsH	3.35	2.97E-02
SMU.702c	putative transcriptional regulator		3.34	2.84E-05
SMU.971	putative 2-amino-4-hydroxy-6-hydroxymethylpteridine pyrophosphokinase	folK	3.3	2.13E-02
SMU.1837	putative DAHP synthase; phospho-2-dehydro-3-deoxyphosphoheptonate aldolase	aroH	3.28	7.39E-03
SMU.1316c	hypothetical protein		3.28	2.91E-05
SMU.1622	methionine sulfoxide reductase A	pmsR	3.26	5.06E-03
SMU.984	hypothetical protein		3.19	6.01E-03
SMU.1909c	hypothetical protein		3.16	8.39E-04
SMU.396	putative glycerol uptake facilitator protein	glpF	3.13	2.81E-03
SMU.183	putative Mn/Zn ABC transporter	sloB	3.12	2.31E-02
SMU.555	hypothetical protein	ylmG	3.12	7.87E-06
SMU.849	50S ribosomal protein L27	rpmA	3.11	1.26E-02
SMU.454	putative cell division protein	ftsL	3.09	4.71E-03
SMU.1227	putative purine nucleoside phosphorylase	deoD	3.09	3.58E-04
SMU.1865	putative A/G-specific DNA glycosylase	mutY	3.09	1.81E-02
SMU.668c	ribonucleotide--diphosphate reductase subunit alpha		3.06	2.24E-05
SMU.2135c	30S ribosomal protein S4	rpsD	3.06	3.34E-02
SMU.1718	putative glutamate racemase	murI	3.01	5.27E-03
SMU.1922	putative chromosome replication protein	dnaB	2.94	1.92E-06
SMU.928	putative histidine kinase		2.94	4.60E-05
SMU.1414c	hypothetical protein		2.94	1.26E-02
SMU.1250c	hypothetical protein		2.93	3.50E-03
SMU.1554c	hypothetical protein		2.93	2.70E-03
SMU.1322	acetoin dehydrogenase	budC	2.93	4.29E-03
SMU.1697c	hypothetical protein		2.93	6.65E-07
SMU.1467	adenine phosphoribosyltransferase	apt	2.93	5.05E-04
SMU.410	putative transcriptional regulator	brpA	2.92	3.55E-03
SMU.669c	putative glutaredoxin		2.91	2.26E-04
SMU.182	iron/manganese ABC transporter ATP-binding protein	sloA	2.9	4.31E-04
SMU.1435c	hypothetical protein		2.89	2.94E-02
SMU.1913c	putative immunity protein, BLpL-like		2.88	2.26E-02

Table 3. (Continued)

SMU.992	hypothetical protein		2.87	5.06E-03
SMU.948	hypothetical protein		2.87	6.75E-05
SMU.1948	preprotein translocase subunit	secE	2.86	2.31E-02
SMU.1869	putative thioredoxin	trxA	2.85	5.22E-04
SMU.1852	putative magnesium/cobalt transport protein		2.84	5.55E-03
SMU.1065c	putative transcriptional regulator (GntR family)		2.84	9.61E-03
SMU.22	putative secreted antigen GbpB/SagA; putative peptidoglycan hydrolase	gbpB	2.83	1.14E-02
SMU.965	homoserine dehydrogenase		2.83	1.94E-06
SMU.717	putative peptidoglycan branched peptide synthesis protein	murM	2.8	1.96E-05
SMU.1786	putative undecaprenyl pyrophosphate synthetase	uppS	2.79	2.32E-03
SMU.596	phosphoglyceromutase	pmgY	2.79	1.07E-03
SMU.2159	putative ABC transporter, ATP-binding protein		2.79	3.17E-02
SMU.830	RgpFc protein	rgpF	2.78	6.42E-07
SMU.2161c	hypothetical protein		2.76	6.79E-04
SMU.769	hypothetical protein		2.75	1.05E-02
SMU.2093	putative transcriptional regulator of arginine metabolism	argR	2.74	4.59E-03
SMU.1996	putative isopentenyl monophosphate kinase, ipk	ipk	2.74	2.05E-03
SMU.1563	putative cation-transporting P-type ATPase PacL, pacL	pacL	2.73	3.03E-03
SMU.841	putative aminotransferase		2.72	7.36E-03
SMU.1945	hypothetical protein		2.71	6.26E-03
SMU.15	putative cell division protein FtsH, ftsH	ftsH	2.69	4.94E-02
SMU.1411	hypothetical protein		2.68	4.88E-05
SMU.1773c	hypothetical protein		2.67	1.06E-05
SMU.921	putative transcriptional regulator		2.67	1.59E-04
SMU.455	putative penicillin-binding protein 2X, pbp2x	pbp2x	2.66	4.30E-02
SMU.268	adenylosuccinate synthetase	purA	2.65	3.20E-02
SMU.1402c	hypothetical protein		2.63	2.67E-04
SMU.853	putative lipoprotein signal peptidase, lspA	lspA	2.62	2.97E-02
SMU.824	dTDP-4-keto-L-rhamnose reductase		2.62	5.71E-06
SMU.1823	putative pyrazinamidase/nicotinamidase	plcA	2.62	1.79E-02
SMU.2164	serine protease	gtrA	2.61	3.88E-05
SMU.1729c	putative aminodeoxychorismate lyase (fragment)		2.6	7.55E-06
SMU.1949	putative membrane carboxypeptidase, penicillin-binding protein 2a	pbp2a	2.6	1.58E-02
SMU.1871c	hypothetical protein		2.6	6.17E-07
SMU.949	ATP-dependent protease Clp, ATPase subunit ClpX	clpX	2.6	6.88E-03
SMU.321	hypothetical protein; possible membrane protein		2.59	2.48E-02
SMU.1192	DNA polymerase III, alpha chain	dnaE	2.59	3.81E-02
SMU.1191	6-phosphofructokinase	pfk	2.59	1.61E-05
SMU.1858	30S ribosomal protein S18	rpsR	2.59	9.83E-03
SMU.860	carbamoylphosphate synthetase, large subunit	carB	2.58	2.71E-03
SMU.08	putative transcription-repair coupling factor	trcF	2.55	9.29E-04
SMU.553	hypothetical protein	ylmE	2.55	1.07E-05
SMU.892	putative type I restriction-modification system, specificity determinant; restriction endonuclease	hsdS	2.54	1.86E-02
SMU.688	hypothetical protein		2.53	1.34E-02
SMU.277	hypothetical protein		2.52	1.48E-04
SMU.1736	acetyl-CoA carboxylase biotin carboxylase subunit	accC	2.52	3.81E-04
SMU.1324	putative cell-division protein	ftsX	2.5	3.15E-04
SMU.991	putative ribonucleotide reductase		2.5	9.37E-04
SMU.1396	glucan-binding protein C	gbpC	2.5	2.00E-04

Table 3. (Continued)

SMU.1188	putative signal peptidase	lepB	2.49	2.91E-06
SMU.1850	putative aminopeptidase	pepP	2.49	2.12E-05
SMU.91	peptidyl-prolyl isomerase RopA (trigger factor)	ropA	2.48	2.94E-02
SMU.244	undecaprenyl pyrophosphate phosphatase	uppP	2.47	1.85E-03
SMU.1573	putative S-adenosylmethionine synthetase	metK	2.46	7.64E-04
SMU.827	putative polysaccharide ABC transporter, permease protein	rgpC	2.45	2.04E-06
SMU.287	putative ComB, accessory factor for ComA	comB	2.44	2.39E-04
SMU.1276c	putative septation ring formation regulator EzrA		2.44	5.41E-03
SMU.336	putative ribonuclease P protein component	rnpA	2.44	3.17E-06
SMU.2079c	hypothetical protein		2.42	2.59E-02
SMU.2077c	hypothetical protein		2.42	1.95E-02
SMU.1847	translation elongation factor P	efp	2.41	2.87E-02
SMU.966	homoserine kinase		2.41	4.82E-03
SMU.359	elongation factor G	fusA	2.4	2.42E-02
SMU.1654c	putative acetyltransferase		2.39	2.42E-05
SMU.1097c	putative transcriptional regulator protein		2.39	2.52E-02
SMU.1992	putative tyrosyl-tRNA synthetase	tyrS	2.39	1.39E-02
SMU.610	cell surface antigen	spaP	2.39	1.83E-05
SMU.1064c	GntR family transcriptional regulator		2.38	9.62E-04
SMU.770c	putative manganese transporter		2.37	7.48E-03
SMU.1377c	hypothetical protein		2.37	2.43E-04
SMU.469	Holliday junction-specific endonuclease	recU	2.36	1.38E-02
SMU.1247	phosphopyruvate hydratase	eno	2.36	2.64E-03
SMU.1189c	hypothetical protein		2.35	1.13E-02
SMU.1100c	putative permease		2.34	1.11E-03
SMU.219	hypothetical protein		2.34	1.80E-05
SMU.415	hypothetical protein		2.33	1.09E-03
SMU.1176	putative cation efflux transporter		2.33	2.89E-06
SMU.630	hypothetical protein		2.33	2.35E-02
SMU.632	putative transcriptional regulator		2.31	1.25E-03
SMU.983	putative transcriptional regulator	bglC	2.31	1.51E-03
SMU.800	hypothetical protein		2.3	4.01E-02
SMU.974	putative spermidine/putrescine ABC transporter, permease protein	potB	2.29	2.86E-03
SMU.1783	putative prolyl-tRNA synthetase	proS	2.29	1.54E-02
SMU.629	putative manganese-type superoxide dismutase, Fe/Mn-SOD	sod	2.27	2.03E-02
SMU.228	putative alkaline-shock protein		2.27	1.10E-03
SMU.2140c	hypothetical protein		2.26	1.79E-04
SMU.1638c	hypothetical protein		2.26	2.06E-02
SMU.1517	putative response regulator CovR; VicR homolog	vicR	2.25	2.66E-02
SMU.1409c	putative transcriptional regulator		2.24	8.61E-03
SMU.2157	inosine monophosphate dehydrogenase	guaB	2.23	3.14E-02
SMU.635	hypothetical protein		2.22	3.19E-02
SMU.1317c	hypothetical protein		2.21	3.15E-02
SMU.1178	putative amino acid ABC transporter, ATP-binding protein		2.2	1.76E-02
SMU.1709	putative potassium uptake protein	trkH	2.2	1.82E-03
SMU.341	putative deoxyribonuclease		2.19	1.24E-02
SMU.1348	putative ABC transporter, ATP-binding protein		2.17	4.85E-03
SMU.16	putative amino acid permease		2.16	8.09E-04
SMU.950	GTP-binding protein	engB	2.15	8.46E-05
SMU.1203	putative branched-chain amino acid aminotransferase	ilvE	2.14	3.04E-07
SMU.1609c	preprotein translocase subunit secG	secG	2.14	3.68E-03

Table 3. (Continued)

SMU.23	ribose-phosphate pyrophosphokinase	prs	2.13	3.21E-02
SMU.1748	aspartokinase	akh	2.13	2.79E-02
SMU.552	cell division protein	ftsZ	2.13	1.84E-04
SMU.759	putative protease		2.13	1.65E-04
SMU.701c	hypothetical protein; putative integral membrane protein		2.12	1.88E-04
SMU.614	hypothetical protein		2.11	5.12E-03
SMU.776	hypothetical protein		2.11	4.80E-05
SMU.944	thymidylate synthase	thyA	2.1	8.24E-03
SMU.482	putative RNA-binding Sun protein; rRNA methylase	sunL	2.09	4.40E-03
SMU.1066	bifunctional GMP synthase/glutamine amidotransferase protein	guaA	2.09	1.41E-06
SMU.1169c	putative thioredoxin family protein		2.09	2.00E-02
SMU.1728	transcription elongation factor	greA	2.08	9.92E-03
SMU.1499	putative exonuclease	rexA	2.08	1.61E-03
SMU.506	putative type II restriction endonuclease		2.07	3.50E-05
SMU.1977c	putative transcriptional regulator		2.07	2.22E-02
SMU.1315c	putative ATP-binding protein		2.05	4.50E-04
SMU.1930	putative cytoplasmic membrane protein, lemA-like	lemA	2.05	6.96E-03
SMU.823	hypothetical protein		2.05	4.73E-02
SMU.591c	hypothetical protein		2.04	1.51E-04
SMU.1753c	hypothetical protein		2.04	2.75E-02
SMU.1211	putative glycerol-3-phosphate acyltransferase PlsY		2.04	1.45E-03
SMU.1062	putative ABC transporter, proline/glycine betaine permease protein	opuAb	2.03	1.65E-04
SMU.1838	preprotein translocase subunit SecA	secA	2.03	1.74E-02
SMU.2118	putative ABC transporter; osmoprotectant-binding protein, glycine betaine/carnitine/choline ABC transporter	opuCc	2.02	4.40E-02
SMU.1721c	putative diaminopimelate decarboxylase		2.01	1.20E-03
SMU.151	hypothetical protein		2.01	1.49E-05
SMU.1286c	putative permease; multidrug efflux protein		2.01	3.37E-02
SMU.1204	DNA topoisomerase IV, subunit A	parC	2.01	6.78E-04
SMU.547	hypothetical protein		2	9.55E-05
SMU.522	hypothetical protein		2	2.64E-06
SMU.307	glucose-6-phosphate isomerase	pgi	2	4.73E-02
	down-regulated genes			
SMU.574c	putative membrane protein	lrgB	0.01	3.56E-14
SMU.575c	putative membrane protein	lrgA	0.02	2.25E-02
SMU.1421	branched-chain alpha-keto acid dehydrogenase subunit E2	pdhC	0.03	1.22E-02
SMU.493	formate acetyltransferase (pyruvate formate-lyase 2)	pfl2	0.03	2.99E-02
SMU.494	fructose-6-phosphate aldolase		0.04	1.92E-02
SMU.1403c	hypothetical protein		0.04	1.09E-02
SMU.197c	hypothetical protein		0.04	5.05E-03
SMU.206c	hypothetical protein		0.06	1.24E-02
SMU.1752c	hypothetical protein		0.06	1.75E-02
SMU.1405c	hypothetical protein		0.07	2.61E-03
SMU.1774c	hypothetical protein		0.07	4.39E-03
SMU.609	putative 40K cell wall protein precursor		0.08	2.71E-02
SMU.30	putative phosphoribosylformylglycinamidine synthase, (FGAM synthase)	purL	0.08	1.59E-03
SMU.179	hypothetical protein		0.09	1.73E-02
SMU.448	hypothetical protein		0.1	3.07E-02
SMU.1536	glycogen synthase	glgA	0.1	3.17E-02
SMU.51	phosphoribosylaminoimidazole carboxylase, ATPase subunit	purK	0.1	4.88E-03

Table 3. (Continued)

SMU.1116c	hypothetical protein		0.11	1.66E-02
SMU.1141c	hypothetical protein		0.11	2.71E-02
SMU.360	extracellular glyceraldehyde-3-phosphate dehydrogenase	gapC	0.11	4.75E-02
SMU.188c	HSP33-like chaperonin	hslO	0.12	3.64E-07
SMU.234	threonine dehydratase, ilvA	ilvA	0.12	4.49E-02
SMU.1754c	hypothetical protein		0.12	2.49E-02
SMU.1538	glucose-1-phosphate adenylyltransferase	glgC	0.12	3.04E-02
SMU.1782	hypothetical protein		0.13	6.50E-06
SMU.1757c	hypothetical protein		0.13	1.29E-02
SMU.198c	putative conjugative transposon protein		0.17	3.68E-03
SMU.272	putative PTS system, enzyme IIA component	ptxA	0.17	2.89E-02
SMU.402	pyruvate formate-lyase	pfl	0.17	2.36E-04
SMU.1346	putative thioesterase	bacT	0.18	4.01E-02
SMU.1961c	putative PTS system, sugar-specific enzyme IIA component		0.19	1.16E-02
SMU.1596	cellobiose phosphotransferase system IIC component	celD	0.19	1.19E-02
SMU.1037c	putative histidine kinase		0.2	4.23E-02
SMU.1263	putative phosphoribosyl-ATP pyrophosphatase / phosphoribosyl-AMP cyclohydrolase	hisI	0.2	2.54E-02
SMU.963c	hypothetical protein; putative deacetylase		0.21	2.42E-02
SMU.18	hypothetical protein		0.21	4.79E-04
SMU.625	putative competence protein	comEA	0.21	3.21E-02
SMU.1602	putative NAD(P)H-flavin oxidoreductase		0.21	7.17E-04
SMU.1582c	hypothetical protein		0.22	2.05E-07
SMU.872	putative PTS system, fructose-specific enzyme IIABC component		0.22	6.49E-03
SMU.226c	putative transposase		0.23	1.48E-02
SMU.1601	6-phospho-beta-glucosidase	celA	0.23	2.31E-02
SMU.886	galactokinase	galK	0.23	1.48E-04
SMU.1878	putative PTS system, mannose-specific component IIC	ptnC	0.23	1.10E-05
SMU.28	putative ATP-binding protein		0.23	7.60E-04
SMU.180	putative oxidoreductase; possible fumarate reductase		0.24	1.17E-02
SMU.441	putative transcriptional regulator		0.24	1.33E-04
SMU.2050c	ribosomal protein L11 methyltransferase	prmA	0.24	2.59E-02
SMU.566c	hypothetical protein		0.24	1.01E-02
SMU.1086	thymidine kinase	kitH	0.24	3.60E-02
SMU.500	putative ribosome-associated protein		0.25	3.07E-02
SMU.271	putative PTS system, enzyme IIB component	ptxB	0.25	2.17E-03
SMU.37	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	purH	0.25	1.83E-04
SMU.1578	biotin--protein ligase	birA	0.25	8.61E-03
SMU.195c	hypothetical protein		0.26	5.44E-09
SMU.1987	putative ABC transporter, ATP-binding protein ComYA; late competence gene	comYA	0.26	4.05E-03
SMU.1760c	hypothetical protein		0.27	3.65E-06
SMU.106c	putative transposase fragment		0.27	1.75E-03
SMU.1020	putative citrate lyase CilB, citryl-CoA lyase, beta subunit	cilB	0.27	2.46E-02
SMU.1737	3(R)-hydroxymyristoyl-(acyl carrier protein) dehydratase	fabZ	0.28	3.46E-03
SMU.52	hypothetical protein		0.28	4.32E-06
SMU.442	hypothetical protein		0.28	4.41E-03
SMU.273	3-keto-L-gulonate-6-phosphate decarboxylase		0.28	8.60E-03
SMU.1069c	hypothetical protein		0.28	2.57E-02
SMU.216c	hypothetical protein		0.28	2.87E-02

Table 3. (Continued)

SMU.959c	hypothetical protein		0.28	1.86E-02
SMU.212c	hypothetical protein		0.28	1.25E-04
SMU.1569	putative maltose/maltodextrin ABC transporter, permease protein	malF	0.29	1.38E-07
SMU.1585c	putative transcriptional regulator		0.29	2.42E-02
SMU.883	dextran glucosidase	dexB	0.29	4.25E-04
SMU.82	molecular chaperone	Dnak	0.29	8.79E-03
SMU.1633c	hypothetical protein		0.29	1.29E-02
SMU.1681c	hypothetical protein		0.29	1.67E-02
SMU.1618	diacylglycerol kinase	dagK	0.3	3.68E-02
SMU.1896c	hypothetical protein		0.3	2.28E-04
SMU.311	PTS system, sorbitol (glucitol) phosphotransferase enzyme IIC2		0.3	2.02E-03
SMU.274	putative hexulose-6-phosphate isomerase		0.3	7.21E-05
SMU.1958c	putative PTS system, mannose-specific IIC component		0.32	1.98E-02
SMU.1615c	hypothetical protein		0.32	3.94E-02
SMU.1067c	putative ABC transporter, permease protein		0.32	1.12E-02
SMU.1695	molybdenum ABC transporter ATP-binding protein		0.32	2.69E-02
SMU.843	hypothetical protein		0.33	4.07E-03
SMU.621c	hypothetical protein		0.33	3.43E-06
SMU.1898	putative ABC transporter, ATP-binding and permease protein		0.33	9.73E-05
SMU.1522	putative amino acid ABC transporter, integral membrane protein	glnP	0.33	6.70E-04
SMU.1073	formate--tetrahydrofolate ligase	fthS	0.33	1.80E-07
SMU.1363c	putative transposase		0.33	4.73E-02
SMU.1019	citrate lyase, gamma-subunit	cilG	0.34	3.55E-02
SMU.491	putative DeoR-type transcriptional regulator		0.35	4.40E-02
SMU.873	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase	metE	0.35	1.32E-02
SMU.191	putative integrase		0.35	2.98E-02
SMU.137	malate dehydrogenase	mleS	0.35	4.86E-03
SMU.207c	putative transposon protein		0.36	3.25E-02
SMU.49	hypothetical protein		0.37	6.65E-03
SMU.421	translation initiation factor 2	infB	0.37	1.72E-07
SMU.1279c	putative cell division protein (cell shape determining protein)		0.37	8.40E-04
SMU.2019	50s ribosomal protein L29	rpmC	0.37	1.04E-02
SMU.1957	putative PTS system, mannose-specific IID component		0.38	7.40E-04
SMU.498	putative late competence protein	comF	0.38	4.16E-02
SMU.34	phosphoribosylaminoimidazole synthetase	purM	0.38	3.54E-02
SMU.32	amidophosphoribosyl transferase	purF	0.39	8.02E-04
SMU.1389	hypothetical protein, pckA	pckA	0.39	2.79E-05
SMU.1892c	hypothetical protein		0.39	1.83E-05
SMU.119	putative alcohol dehydrogenase class III	adh	0.4	3.82E-07
SMU.573	hypothetical protein		0.4	1.36E-05
SMU.1960c	putative PTS system, mannose-specific IIB component		0.4	4.20E-03
SMU.1089	hypothetical protein		0.4	8.62E-05
SMU.1256c	hypothetical protein		0.4	4.24E-02
SMU.422	ribosome binding factor A	rbfA	0.41	3.18E-05
SMU.1614	formamidopyrimidine-DNA glycosylase	fpg	0.41	1.15E-02
SMU.1489	hypothetical protein, LacX		0.41	2.38E-03
SMU.1556	methionine aminopeptidase	ampM	0.41	1.91E-02
SMU.529	hypothetical protein		0.41	1.01E-03
SMU.1821c	aspartyl/glutamyl-tRNA amidotransferase subunit C	gatC	0.42	9.64E-03
SMU.2054c	hypothetical protein		0.42	1.04E-04

Table 3. (Continued)

SMU.1818c	hypothetical protein		0.42	4.80E-04
SMU.532	anthranilate synthase component I	trpE	0.42	4.52E-03
SMU.2029	class III stress response-related ATP-dependent Clp protease, ATP-binding subunit	clpC	0.42	1.99E-02
SMU.1562	putative potassium uptake protein TrkA	trk	0.43	2.29E-04
SMU.1523	putative membrane nuclease	endA	0.43	4.12E-02
SMU.754	HPr (serine) kinase/phosphatase		0.43	1.68E-03
SMU.346	putative NADH dehydrogenase; NAD(P)H nitroreductase		0.43	2.12E-02
SMU.404c	hypothetical protein		0.43	1.61E-02
SMU.1927	putative ABC transporter, ATP-binding protein		0.43	1.37E-02
SMU.35	phosphoribosylglycinamide formyltransferase	punN	0.43	4.90E-02
SMU.490	putative pyruvate formate-lyase activating enzyme	pflC	0.43	1.86E-02
SMU.1337c	putative alpha/beta superfamily hydrolase		0.43	1.75E-02
SMU.677	MerR family transcriptional regulator		0.44	1.90E-02
SMU.1352	putative transposase		0.44	1.29E-02
SMU.92c	putative transposase		0.44	1.45E-02
SMU.1456	hypothetical protein		0.44	1.67E-03
SMU.2076c	hypothetical protein		0.45	4.73E-02
SMU.534	anthranilate phosphoribosyltransferase	trpD	0.45	2.23E-05
SMU.1907	hypothetical protein		0.45	1.06E-02
SMU.641	putative oxidoreductase		0.45	1.76E-02
SMU.1269	putative phosphoserine phosphatase	serB	0.45	4.19E-02
SMU.1272	putative histidyl-tRNA synthetase	hisZ	0.46	5.43E-03
SMU.387	O-syaloglycoprotein endopeptidase		0.46	3.69E-02
SMU.2058	putative transcriptional regulator		0.46	3.55E-02
SMU.138	putative malate permease		0.46	9.82E-03
SMU.537	putative tryptophan synthase, beta subunit	trpB	0.47	2.77E-04
SMU.1117	NADH oxidase (H2O-forming)	naoX	0.47	3.65E-03
SMU.1840	putative fructokinase	scrK	0.47	8.11E-03
SMU.1335c	putative enoyl-(acyl-carrier-protein) reductase		0.48	2.12E-03
SMU.1022	2'-(5"-triphosphoribosyl)-3'-dephospho-CoA:apo-citrate lyase	citX	0.48	2.40E-02
SMU.982	putative BglB fragment	bglB2	0.48	1.96E-04
SMU.1225	putative transcriptional regulator	cpsY	0.48	2.03E-06
SMU.248	putative ABC transporter, membrane protein		0.49	1.29E-05
SMU.1164c	putative ABC transporter, ATP-binding protein		0.49	4.62E-02
SMU.54	putative amino acid recemase		0.49	1.28E-02
SMU.1851	excinuclease ABC subunit A	uvrA	0.49	1.20E-04
SMU.2065	putative UDP-glucose 4-epimerase		0.5	8.26E-07
SMU.664	bifunctional ornithine acetyltransferase/N-acetylglutamate synthase	argJ	0.5	1.83E-03
SMU.1805	putative transcriptional regulator		0.5	3.26E-02
SMU.1542c	putative lipid kinase		0.5	3.83E-03

Table 4. Differentially expressed genes in "fed" biofilms *vs.* "restarved" biofilms (p-value<0.05).

Ratio represents the value for each differentially expressed gene in "restarved" biofilms.

Locus_tag	Description	Name	Ratio	p-value
	up-regulated genes			
SMU.2057c	putative cadmium-transporting ATPase		63.96	5.67E-03
SMU.575c	putative membrane protein	lrgA	37.33	3.76E-02
SMU.609	putative 40K cell wall protein precursor		32.19	2.92E-02
SMU.1405c	hypothetical protein		28.24	2.35E-02
SMU.1520	putative ABC transporter, glutamine binding protein		21.09	4.50E-02
SMU.1538	glucose-1-phosphate adenylyltransferase	glgC	20.49	3.53E-02
SMU.574c	putative membrane protein	lrgB	19.76	1.49E-02
SMU.1539	1,4-alpha-glucan branching enzyme	glgB	19.68	6.80E-02
SMU.871	putative fructose-1-phosphate kinase	pfkB	14.29	2.68E-09
SMU.1547c	putative response regulator		13.85	6.39E-03
SMU.870	putative transcriptional regulator of sugar metabolism		12.42	4.66E-05
SMU.1564	putative glycogen phosphorylase	glgP	11.67	2.69E-03
SMU.1536	glycogen synthase	glgA	11.43	1.28E-02
SMU.402	pyruvate formate-lyase	pfl	11.28	2.13E-02
SMU.1589c	putative hexosyltransferase		10.8	1.54E-03
SMU.1177c	putative ABC transporter, glutamine binding protein		10.27	6.31E-03
SMU.872	putative PTS system, fructose-specific enzyme IIABC component		10.05	3.79E-03
SMU.270	ascorbate-specific PTS system enzyme IIC	ulaA	10.03	4.48E-03
SMU.179	hypothetical protein		9.27	5.00E-03
SMU.879	multiple sugar-binding ABC transporter, permease protein	msmF	8.94	4.77E-07
SMU.878	multiple sugar-binding ABC transporter	msmE	8.15	3.06E-03
SMU.180	putative oxidoreductase; fumarate reductase		7.7	3.31E-03
SMU.1960c	putative PTS system, mannose-specific IIB component		7.67	5.90E-04
SMU.882	multiple sugar-binding ABC transporter, ATP-binding protein	msmK	7.65	8.35E-07
SMU.2047	putative PTS system, glucose-specific IIABC component	ptsG	7.15	5.22E-03
SMU.1961c	putative PTS system, sugar-specific enzyme IIA component		7.07	1.73E-02
SMU.1582c	hypothetical protein		6.72	2.34E-09
SMU.1141c	hypothetical protein		6.61	2.17E-02
SMU.1878	putative PTS system, mannose-specific component IIC	ptnC	6.6	7.77E-03
SMU.1077	putative phosphoglucomutase	pgm	6.51	6.17E-02
SMU.1596	putative PTS system, cellobiose phosphotransferase system IIC component	celD	6.25	3.41E-02
SMU.493	formate acetyltransferase (pyruvate formate-lyase 2)	pfl2	6.23	6.34E-02
SMU.1956c	hypothetical protein		6.23	5.85E-07
SMU.494	fructose-6-phosphate aldolase		5.17	5.87E-02
SMU.50	phosphoribosylaminoimidazole carboxylase, catalytic subunit	purE	5.05	8.63E-03
SMU.1089	hypothetical protein		4.91	4.25E-03
SMU.883	dextran glucosidase	dexB	4.9	1.37E-04
SMU.1908c	hypothetical protein		4.89	3.74E-02
SMU.1879	putative PTS system, mannose-specific component IID		4.85	1.13E-03
SMU.1957	putative PTS system, mannose-specific IID component		4.81	1.77E-03
SMU.1487	hypothetical protein		4.62	3.88E-02
SMU.1116c	hypothetical protein		4.57	3.74E-05
SMU.104	putative alpha-glucosidase; glycosyl hydrolase		4.48	6.53E-02
SMU.500	putative ribosome-associated protein		4.47	7.72E-04
SMU.78	fructan hydrolase; exo-beta-D-fructosidase; fructanase	fruA	4.37	5.17E-03
SMU.404c	hypothetical protein		4.35	5.60E-04

Table 4. (Continued)

SMU.1489	hypothetical protein	lacX	4.31	5.20E-03
SMU.148	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	adhE	4.23	9.52E-02
SMU.1912c	hypothetical protein		4.18	1.11E-01
SMU.1958c	putative PTS system, mannose-specific IIC component		4.15	2.22E-03
SMU.1843	sucrose-6-phosphate hydrolase	scrB	4.13	1.69E-02
SMU.1021	putative citrate lyase, alfa subunit	cilA	4.12	3.92E-06
SMU.1865	putative A/G-specific DNA glycosylase	mutY	3.98	2.18E-02
SMU.105	putative transcriptional regulator; repressor of sugar transport operon		3.96	1.93E-02
SMU.1904c	hypothetical protein		3.95	6.55E-05
SMU.1335c	putative enoyl-(acyl-carrier-protein) reductase		3.95	6.15E-02
SMU.273	hexulose-6-phosphate synthase	ulaD	3.91	9.68E-03
SMU.2127	putative succinate semialdehyde dehydrogenase		3.87	1.96E-02
SMU.1877	putative PTS system, mannose-specific component IIAB	ptnA	3.84	5.76E-03
SMU.272	putative PTS system, enzyme IIA component	ptxA	3.77	8.93E-03
SMU.2050c	ribosomal protein L11 methyltransferase	prmA	3.76	2.36E-07
SMU.274	putative hexulose-6-phosphate isomerase		3.74	3.96E-03
SMU.534	putative phosphoribosyl anthranilate transferase	trpD	3.7	3.07E-02
SMU.1020	putative citrate lyase CilB, citryl-CoA lyase, beta subunit	cilB	3.7	1.79E-03
SMU.1681c	hypothetical protein		3.64	7.11E-03
SMU.2035	bacteriocin immunity protein		3.63	1.95E-02
SMU.103	putative PTS system, IIA component		3.62	6.50E-02
SMU.1424	putative dihydrolipoamide dehydrogenase	pdhD	3.51	2.16E-02
SMU.1679c	hypothetical protein		3.49	1.56E-05
SMU.1903c	hypothetical protein		3.47	6.97E-04
SMU.1019	citrate lyase subunit gamma	cilG	3.47	4.33E-02
SMU.1760c	hypothetical protein		3.46	3.04E-02
SMU.1906c	hypothetical protein		3.4	1.78E-03
SMU.1182	mannitol-1-phosphate dehydrogenase	mtlD	3.36	6.30E-04
SMU.102	putative PTS system, IID component		3.34	4.84E-02
SMU.536	phosphoribosyl anthranilate isomerase	trpF	3.34	5.73E-02
SMU.55	hypothetical protein		3.34	3.68E-03
SMU.1601	6-phospho-beta-glucosidase	celA	3.31	3.86E-07
SMU.1905c	putative bacteriocin secretion protein		3.3	9.60E-03
SMU.1844	sucrose operon repressor	scrR	3.27	1.59E-07
SMU.963c	putative deacetylase		3.27	1.02E-06
SMU.1737	putative 3-hydroxymyristoyl-(acyl carrier protein) dehydratase	fabZ	3.24	2.07E-02
SMU.1262c	hypothetical protein		3.23	3.93E-03
SMU.537	tryptophan synthase, beta subunit	trpB	3.22	1.08E-02
SMU.1936c	hypothetical protein		3.21	9.00E-03
SMU.53	hypothetical protein, carboxamide ribonucleotide isomerase		3.17	1.03E-02
SMU.1264	putative imidazoleglycerol-phosphate synthase, cyclase subunit	hisF	3.12	2.52E-03
SMU.1336	hypothetical protein, involved in polyketide synthesis	pksD	3.11	8.49E-04
SMU.67	putative acyltransferase		3.1	5.65E-02
SMU.1735	putative acetyl-CoA carboxylase beta subunit	accD	3.09	8.95E-07
SMU.448	hypothetical protein		3.05	1.10E-01
SMU.1472	putative single-strand DNA-specific exonuclease	recJ	3.03	5.71E-04
SMU.2049c	hypothetical protein		3.03	8.03E-03
SMU.1265	putative phosphoribosyl formimino-5-aminoimidazole	hisA	3.01	2.64E-02
SMU.166	hypothetical protein		2.99	2.59E-02
SMU.533	anthranilate synthase, beta subunit	trpG	2.99	5.25E-02
SMU.1664c	putative acetoin utilization protein, acetoin dehydrogenase		2.98	7.34E-03

Table 4. (Continued)

SMU.1261c	putative phosphoribosyl-ATP pyrophosphohydrolase		2.97	1.39E-05
SMU.844	hypothetical protein		2.97	1.86E-04
SMU.153	hypothetical protein		2.95	1.75E-01
SMU.1611c	putative permease; possible multi-drug resistance efflux pump		2.95	7.03E-02
SMU.1855	hypothetical protein		2.95	6.72E-06
SMU.52	hypothetical protein		2.94	7.44E-02
SMU.51	phosphoribosylaminoimidazole carboxylase, ATPase subunit	purK	2.93	2.62E-03
SMU.1235	tRNA modification GTPase	trmE	2.9	3.70E-07
SMU.528c	hypothetical protein		2.9	1.88E-02
SMU.1363c	putative transposase		2.86	1.34E-02
SMU.2112	glucan-binding protein A	gbpA	2.85	8.87E-02
SMU.2052c	hypothetical protein		2.85	3.15E-03
SMU.1614	formamidopyrimidine-DNA glycosylase	fpG	2.82	7.19E-08
SMU.2128	dihydroxy-acid dehydratase		2.81	4.33E-03
SMU.797	hypothetical protein		2.8	1.63E-07
SMU.1334	putative phosphopantetheinyl transferase	sfp	2.79	6.72E-04
SMU.1268	imidazoleglycerol-phosphate dehydratase	hisB	2.78	1.52E-06
SMU.532	anthranilate synthase, alpha subunit	trpE	2.78	4.84E-02
SMU.1757c	hypothetical protein		2.78	2.05E-03
SMU.1774c	hypothetical protein		2.75	5.78E-09
SMU.1260c	hypothetical protein		2.75	3.00E-02
SMU.809	excinuclease ABC subunit B	uvrB	2.74	2.99E-02
SMU.1910c	hypothetical protein		2.73	9.68E-02
SMU.1017	putative oxaloacetate decarboxylase, sodium ion pump subunit	oadB	2.71	4.59E-04
SMU.1909c	hypothetical protein		2.7	1.97E-02
SMU.1389	hypothetical protein	pckA	2.7	2.98E-07
SMU.1221	orotate phosphoribosyltransferase	pyrE	2.68	2.78E-06
SMU.1754c	hypothetical protein		2.67	4.47E-06
SMU.662	hypothetical protein; possible membrane protein		2.67	1.97E-06
SMU.843	hypothetical protein		2.66	4.64E-10
SMU.200c	hypothetical protein		2.66	4.58E-04
SMU.1578	biotin operon repressor / biotin-[acetyl-CoA-carboxylase] ligase	birA	2.63	9.92E-02
SMU.1133	putative phosphate transport system regulatory protein	phoU	2.63	4.47E-02
SMU.1593c	putative CDP-diglyceride synthetase		2.62	4.93E-04
SMU.197c	SMU.197c hypothetical protein		2.62	3.42E-02
SMU.173	putative ppGpp-regulated growth inhibitor		2.62	1.94E-02
SMU.1086	thymidine kinase	kitH	2.61	4.40E-02
SMU.54	putative amino acid recemase		2.61	1.05E-03
SMU.1090	hypothetical protein		2.6	3.55E-04
SMU.1273	histidinol-phosphate aminotransferase	hisC	2.59	7.68E-02
SMU.795	esterase		2.58	2.19E-02
SMU.1782	hypothetical protein		2.57	2.59E-02
SMU.426	copper-transporting ATPase; P-type ATPase	copA	2.56	3.57E-02
SMU.1938c	putative ABC transporter, permease protein		2.56	8.10E-02
SMU.271	putative PTS system, enzyme IIB component	ptxB	2.56	2.72E-03
SMU.796	hypothetical protein		2.56	2.80E-02
SMU.1602	putative NAD(P)H-flavin oxidoreductase		2.54	2.19E-02
SMU.168	putative transcriptional regulator		2.54	1.50E-02
SMU.1341c	putative gramicidin S synthetase		2.52	1.64E-01
SMU.1067c	putative ABC transporter, permease protein		2.52	5.04E-02

Table 4. (Continued)

SMU.1037c	putative histidine kinase		2.52	4.18E-02
SMU.1561	putative potassium uptake system protein	trkB	2.52	2.49E-09
SMU.1117	NADH oxidase (H2O-forming)	naoX	2.51	5.97E-06
SMU.1337c	putative alpha/beta superfamily hydrolase		2.5	4.82E-04
SMU.1629c	putative cell division protein; DNA segregation ATPase		2.47	8.48E-02
SMU.1173	putative O-acetylhomoserine sulfhydrylase	cysD	2.45	9.08E-03
SMU.1426c	putative phospho-sugar mutase		2.44	1.96E-01
SMU.1777	putative ribonucleotide reductase protein	nrdI	2.44	4.47E-04
SMU.1256c	hypothetical protein, possible membrane-associated Zn-dependent proteases		2.42	3.87E-03
SMU.1784c	putative Eep protein homolog;		2.42	1.20E-03
SMU.746c	hypothetical protein		2.41	5.95E-03
SMU.257	putative transmembrane protein, permease	oppC	2.4	3.28E-03
SMU.538	tryptophan synthase, alpha subunit	trpA	2.39	2.17E-03
SMU.125	hypothetical protein		2.36	1.75E-02
SMU.352	ribulose-phosphate-3-epimerase		2.36	2.46E-04
SMU.1340	putative surfactin synthetase	bacA2	2.34	1.32E-02
SMU.1016	acetyl-CoA carboxylase, biotin carboxyl carrier subunit	bcc	2.34	2.09E-02
SMU.812	hypothetical protein		2.33	2.41E-02
SMU.1422	putative pyruvate dehydrogenase E1 component beta subunit	pdhB	2.32	1.64E-02
SMU.1088	putative thiamine biosynthesis lipoprotein	apbE	2.32	2.76E-04
SMU.1585c	putative transcriptional regulator		2.32	1.56E-02
SMU.1269	putative phosphoserine phosphatase	serB	2.31	2.00E-02
SMU.1271	ATP phosphoribosyltransferase catalytic subunit	hisG	2.31	5.19E-02
SMU.1172c	hypothetical protein		2.31	3.37E-02
SMU.1494	tagatose-6-phosphate kinase	lacC	2.3	4.57E-02
SMU.2056	recombination factor protein RarA		2.29	1.54E-03
SMU.768c	hypothetical protein		2.29	2.12E-02
SMU.1299c	putative acetate kinase		2.29	1.96E-05
SMU.1272	putative histidyl-tRNA synthetase	hisZ	2.28	2.07E-05
SMU.1543	NAD-dependent DNA ligase	ligA	2.27	1.72E-06
SMU.1227	putative purine nucleoside phosphorylase		2.25	5.36E-03
SMU.542	putative glucose kinase	glk	2.25	3.96E-02
SMU.765	NADH oxidase/alkyl hydroperoxidase reductase peroxide-forming		2.23	7.46E-02
SMU.1933c	cobalt permease		2.23	2.99E-01
SMU.1615c	hypothetical protein		2.23	2.44E-02
SMU.1338c	putative permease; possible multidrug-efflux transporter		2.23	5.47E-02
SMU.1934c	putative cobalt ABC transporter, ATP-binding protein		2.2	6.94E-02
SMU.312	PTS system, sorbitol phosphotransferase enzyme IIBC		2.19	3.10E-02
SMU.441	putative transcriptional regulator		2.19	9.06E-10
SMU.88c	mechanosensitive ion channel		2.18	2.62E-02
SMU.1339	putative bacitracin synthetase	bacD	2.18	1.10E-02
SMU.641	putative oxidoreductase		2.17	2.61E-02
SMU.811	hypothetical protein		2.17	6.38E-02
SMU.167	hypothetical protein		2.17	1.47E-01
SMU.1913c	putative immunity protein, BLpL-like		2.16	2.67E-02
SMU.1692	pyruvate-formate lyase activating enzyme	pflA	2.15	2.48E-02
SMU.204c	hypothetical protein		2.14	5.78E-02
SMU.938	putative phosphomevalonate kinase		2.13	1.27E-01
SMU.2104	integral membrane protein		2.12	6.15E-02

Table 4. (Continued)

SMU.1106c	phosphoglycerate mutase-like protein		2.11	6.56E-04
SMU.1306c	hypothetical protein		2.11	2.40E-05
SMU.1827	putative biotin biosynthesis protein		2.1	2.42E-05
SMU.1432c	putative endoglucanase precursor		2.09	2.29E-02
SMU.778	shikimate 5-dehydrogenase	aroE	2.09	1.86E-02
SMU.248	putative ABC transporter, membrane protein		2.08	3.68E-05
SMU.1038c	putative response regulator		2.08	1.68E-02
SMU.1039c	putative lipopolysaccharide glycosyltransferase		2.05	3.62E-04
SMU.1014	hypothetical protein		2.05	2.35E-04
SMU.1044c	putative pseudouridylate synthase		2.02	8.53E-02
SMU.1586	threonyl-tRNA synthetase	thrS	2.02	4.29E-02
SMU.1233	phosphopentomutase	deoB	2.01	8.23E-02
SMU.940c	putative hemolysin III		2.01	1.37E-01
SMU.1504c	hypothetical protein		2.01	2.44E-02
	down-regulated genes			
SMU.1168	putative transcriptional regulator		0.03	6.78E-04
SMU.753	hypothetical protein		0.07	1.17E-04
SMU.120	50S ribosomal protein L28	rpmB	0.07	2.24E-02
SMU.1502c	hypothetical protein		0.08	7.40E-04
SMU.2000	50S ribosomal protein L17	rplQ	0.08	9.31E-04
SMU.865	30S ribosomal protein S16	rpsP	0.09	2.04E-07
SMU.1924	response regulator GcrR for glucan-binding protein C	gcrR	0.09	1.93E-03
SMU.2001	DNA-directed RNA polymerase, alpha subunit	rpoA	0.1	9.29E-04
SMU.2135c	30S ribosomal protein S4	rpsD	0.11	6.81E-03
SMU.2032	30S ribosomal protein S2	rpsB	0.12	3.18E-04
SMU.2002	30S ribosomal protein S11	rs11	0.12	8.25E-09
SMU.340	50S ribosomal protein L34	rpmH	0.12	3.00E-03
SMU.2003a	50S ribosomal protein L36	rpmJ	0.13	1.42E-04
SMU.1781	hypothetical protein		0.13	1.10E-02
SMU.2077c	hypothetical protein		0.13	8.63E-05
SMU.2004	translation initiation factor IF-1	infA	0.13	1.72E-02
SMU.1646c	hemolysis inducing protein		0.13	1.85E-03
SMU.2003	30S ribosomal protein S13	rpsM	0.14	2.25E-04
SMU.1882c	hypothetical protein		0.15	1.43E-03
SMU.10	hypothetical protein		0.15	4.71E-07
SMU.396	putative glycerol uptake facilitator protein	glpF	0.15	6.31E-03
SMU.357	30S ribosomal protein S12	rpsL	0.15	4.96E-06
SMU.1609c	preprotein translocase subunit secG	secG	0.15	1.58E-05
SMU.1858	30S ribosomal protein S18	rpsR	0.16	3.99E-06
SMU.369c	hypothetical protein		0.16	2.08E-03
SMU.1127	30S ribosomal protein S20	rpsT	0.16	2.63E-03
SMU.2078c	holliday junction resolvase-like protein		0.16	6.71E-03
SMU.1236c	hypothetical protein		0.16	1.92E-04
SMU.697	translation initiation factor IF3	infC	0.17	3.07E-07
SMU.358	30S ribosomal protein S7		0.17	3.37E-03
SMU.1765c	hypothetical protein		0.17	2.99E-03
SMU.1923c	transcriptional regulator NrdR; transcriptional repressor	nrdR	0.17	2.13E-03
SMU.14	hypoxanthine-guanine phosphoribosyltransferase	hprT	0.17	1.86E-04
SMU.1992	tyrosyl-tRNA synthetase	tyrS	0.17	3.21E-03
SMU.1249c	hypothetical protein		0.17	6.87E-08
SMU.846	50S ribosomal protein L21	rplU	0.18	3.48E-03

Table 4. (Continued)

SMU.1948	preprotein translocase subunit SecE	secE	0.18	9.48E-04
SMU.564	hypothetical protein		0.18	5.28E-08
SMU.1527	FoF1 membrane-bound proton-translocating ATPase, epsilon subunit	atpC	0.18	4.90E-03
SMU.1200	30S ribosomal protein S1	rpsA	0.18	1.24E-03
SMU.453	S-adenosyl-methyltransferase	mraW	0.18	3.59E-09
SMU.1859	single-stranded DNA-binding protein	ssb	0.18	2.82E-02
SMU.1467	adenine phosphoribosyltransferase	apt	0.19	5.80E-06
SMU.1947	transcription antitermination factor	nusG	0.19	1.07E-03
SMU.1745c	putative transcriptional regulator		0.2	1.63E-07
SMU.2079c	hypothetical protein		0.2	1.71E-02
SMU.948	hypothetical protein		0.21	1.09E-06
SMU.1288	50S ribosomal protein L19	rplS	0.22	1.37E-02
SMU.958	hypothetical protein		0.22	9.43E-07
SMU.596	phosphoglyceromutase	pmgY	0.22	3.90E-03
SMU.96	DNA-directed RNA polymerase, delta subunit	rpoE	0.23	6.77E-02
SMU.849	50S ribosomal protein L27	rpmA	0.23	3.60E-03
SMU.1287	putative transcriptional regulator		0.23	1.66E-06
SMU.1876	hypothetical protein		0.23	3.42E-02
SMU.2129c	hypothetical protein		0.24	3.85E-07
SMU.1996	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	ipk	0.24	1.01E-03
SMU.848	hypothetical protein		0.24	3.06E-08
SMU.478	guanylate kinase		0.24	1.38E-02
SMU.1724c	putative rRNA methylase		0.24	1.37E-02
SMU.1816	putative maturase-related protein		0.25	5.05E-03
SMU.2084	transcriptional regulator	spxA	0.25	4.07E-03
SMU.2005	adenylate kinase	adk	0.25	7.56E-05
SMU.1061	hypothetical protein	ylxM	0.25	2.30E-04
SMU.589	putative DNA-binding protein		0.26	6.31E-03
SMU.336	ribonuclease P		0.26	1.12E-05
SMU.1746c	enoyl-CoA hydratase		0.26	1.42E-08
SMU.1697c	hypothetical protein		0.26	2.13E-02
SMU.1743	acyl carrier protein	acpP	0.27	9.31E-02
SMU.1860	30S ribosomal protein S6	rpsF	0.27	1.19E-05
SMU.169	50S ribosomal protein L13		0.27	3.82E-04
SMU.2140c	hypothetical protein	rplM	0.27	2.80E-06
SMU.1188	putative signal peptidase	lepB	0.27	6.03E-09
SMU.1703c	hypothetical protein		0.27	8.94E-07
SMU.170	30S ribosomal protein S9	rpsI	0.27	5.97E-06
SMU.957	50S ribosomal protein L10	rplJ	0.28	1.16E-03
SMU.2136c	hypothetical protein		0.28	4.72E-02
SMU.2137c	hypothetical protein		0.28	3.73E-05
SMU.1462c	putative oxidoreductase		0.28	4.09E-04
SMU.1718	putative glutamate racemase	murI	0.29	1.35E-04
SMU.991	putative ribonucleotide reductase		0.29	3.10E-02
SMU.1348c	putative ABC transporter, ATP-binding protein		0.29	3.32E-04
SMU.1258c	restriction endonuclease		0.29	1.93E-02
SMU.530c	hypothetical protein		0.3	7.50E-03
SMU.611	ATP-dependent RNA helicase		0.3	1.59E-02
SMU.1163c	putative ABC transporter, ATP-binding protein		0.3	4.67E-02
SMU.1847	putative translation elongation factor P	efp	0.3	6.67E-05
SMU.1902c	hypothetical protein		0.3	2.07E-03

Table 4. (Continued)

SMU.1671c	hypothetical protein		0.31	5.91E-03
SMU.707c	putative endolysin		0.31	4.44E-06
SMU.1942c	putative amino acid binding protein		0.31	8.83E-02
SMU.1068c	putative ABC transporter, ATP-binding protein		0.31	2.86E-07
SMU.513	hypothetical protein		0.31	4.39E-03
SMU.1852	putative magnesium/cobalt transport protein		0.31	5.43E-06
SMU.1622	methionine sulfoxide reductase A	pmsR	0.31	4.89E-02
SMU.2031	elongation factor Ts	tsf	0.32	1.40E-03
SMU.1047c	hypothetical protein		0.32	8.63E-06
SMU.1475c	hypothetical protein		0.32	1.46E-05
SMU.1691	putative D-alanine-D-alanyl carrier protein ligase		0.32	1.69E-03
SMU.818	30S ribosomal protein S21	rpsU	0.32	3.56E-07
SMU.277	hypothetical protein		0.32	1.29E-02
SMU.2138	replicative DNA helicase	dnaC	0.33	1.45E-03
SMU.333	hypothetical protein		0.33	5.78E-02
SMU.2097	hypothetical protein		0.33	1.04E-07
SMU.1626	50S ribosomal protein L1	rplA	0.34	7.08E-03
SMU.245	adaptor protein	mecA	0.34	1.50E-04
SMU.471	hypothetical protein		0.34	6.00E-04
SMU.475	hypothetical protein		0.34	2.93E-03
SMU.1926	putative transcriptional regulator	psaR	0.35	1.99E-02
SMU.299c	putative bacteriocin peptide precursor		0.35	8.09E-02
SMU.415	hypothetical protein		0.35	1.24E-02
SMU.1729c	putative aminodeoxychorismate lyase (fragment)		0.35	2.63E-06
SMU.1871	hypothetical protein		0.35	5.60E-04
SMU.1192	DNA polymerase III, alpha chain	dnaE	0.35	2.46E-02
SMU.278	hypothetical protein		0.35	2.62E-02
SMU.154	30S ribosomal protein S15	rpsO	0.35	5.62E-02
SMU.2153c	putative peptidase		0.36	2.70E-02
SMU.05	hypothetical protein		0.36	2.84E-07
SMU.1377	hypothetical protein		0.37	2.30E-05
SMU.2164	serine protease	htrA	0.37	4.07E-02
SMU.1289c	putative permease, chloride channel		0.37	6.37E-07
SMU.547	hypothetical protein		0.37	7.85E-03
SMU.1098c	putative oxidoreductase		0.38	5.66E-08
SMU.667	putative ribonucleotide reductase, small subunit		0.38	1.23E-02
SMU.1003	tRNA (uracil-5-)-methyltransferase Gid; glucose inhibited division protein	gid	0.38	4.02E-02
SMU.676	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	gapN	0.38	4.94E-08
SMU.2143c	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase	trmU	0.38	3.64E-03
SMU.567	putative glutamine ABC transporter, permease component		0.38	2.14E-02
SMU.2026c	30S ribosomal protein S10		0.39	2.34E-02
SMU.1189c	hypothetical protein		0.39	4.82E-02
SMU.26	putative fatty acid/phospholipid synthesis protein	plsX	0.39	1.71E-02
SMU.773c	lysyl-tRNA synthetase	lysS	0.39	8.73E-03
SMU.394c	hypothetical protein		0.39	1.36E-03
SMU.1869	putative thioredoxin	trxA	0.39	6.13E-06
SMU.1349	hypothetical protein		0.39	1.05E-02
SMU.698	50S ribosomal protein L35	rpmI	0.40	8.09E-03
SMU.774	hypothetical protein		0.40	2.41E-02

Table 4. (Continued)

SMU.1455	mutator protein, pyrophosphohydrolase	mutX	0.40	1.00E-01
SMU.988	putative cardiolipin synthase		0.40	3.72E-04
SMU.1787c	preprotein translocase subunit	yajC	0.40	2.12E-02
SMU.1922	putative chromosome replication protein	dnaB	0.40	1.08E-03
SMU.1669	putative ABC transporter, branched chain amino acid-binding protein	livK	0.40	5.71E-04
SMU.474	S-ribosylhomocysteinase	luxS	0.40	1.11E-02
SMU.1100c	putative permease		0.40	4.82E-03
SMU.417	hypothetical protein		0.40	1.20E-03
SMU.599	D-alanyl-alanine synthetase A	ddl	0.40	1.18E-09
SMU.1492	PTS system, lactose-specific enzyme IIA EIIA-LAC	lacF	0.41	2.56E-02
SMU.1846c	hypothetical protein		0.41	3.42E-08
SMU.1304c	hypothetical protein		0.41	5.99E-03
SMU.01	chromosomal replication initiator protein, DnaA	dnaA	0.41	5.64E-02
SMU.572	putative tetrahydrofolate dehydrogenase/cyclohydrolase	folD	0.41	1.42E-02
SMU.602	putative sodium-dependent transporter		0.41	7.22E-03
SMU.1323	hydrolase		0.41	5.18E-04
SMU.1278c	hypothetical protein		0.42	3.72E-03
SMU.1324	putative cell-division protein	ftsX	0.42	1.59E-02
SMU.1386	uridine kinase	urk	0.42	1.47E-02
SMU.699	50S ribosomal protein L20	rplT	0.42	1.27E-04
SMU.759	putative protease		0.42	5.64E-07
SMU.1325	putative ABC transporter, ATP-binding component	ftsE	0.42	3.46E-02
SMU.294	hypothetical protein		0.42	1.36E-02
SMU.761	putative protease		0.42	1.14E-04
SMU.23	ribose-phosphate pyrophosphokinase	prs	0.42	4.57E-02
SMU.1931	glucose-inhibited division protein	gidB	0.42	1.46E-02
SMU.2139c	50S ribosomal protein L9	rplI	0.42	1.08E-02
SMU.444	hypothetical protein		0.42	4.29E-03
SMU.1603	putative lactoylglutathione lyase	lguL	0.43	3.16E-02
SMU.367	hypothetical protein		0.43	8.82E-02
SMU.1447c	hypothetical protein		0.43	1.09E-02
SMU.2161	hypothetical protein		0.43	2.11E-03
SMU.1444c	hypothetical protein		0.43	2.56E-02
SMU.344	hypothetical protein		0.43	5.24E-02
SMU.12	hypothetical protein		0.43	2.81E-02
SMU.454	putative cell division protein	ftsL	0.43	1.51E-05
SMU.751	transcriptional accessory protein		0.43	2.71E-03
SMU.1534	FoF1 membrane-bound proton-translocating ATPase, c subunit	atpH	0.44	1.03E-04
SMU.1748	aspartokinase	akh	0.44	3.59E-04
SMU.2105	hypothetical protein		0.44	7.91E-02
SMU.2146c	hypothetical protein		0.44	4.75E-06
SMU.841	putative aminotransferase		0.44	2.99E-02
SMU.2093	putative transcriptional regulator of arginine metabolism	argR	0.44	1.20E-05
SMU.1498	lactose repressor	lacR	0.44	8.54E-05
SMU.924	thiol peroxidase	tpx	0.44	1.03E-04
SMU.1429	putative UDP-N-acetylmuramyl tripeptide synthetase MurC	murC2	0.44	4.62E-05
SMU.218	putative transcriptional regulator		0.45	1.96E-01
SMU.1643c	hypothetical protein		0.45	7.38E-03
SMU.1930	putative cytoplasmic membrane protein; LemA-like protein	lemA	0.45	3.09E-02

Table 4. (Continued)

SMU.1670c	hypothetical protein		0.45	1.34E-02
SMU.690	hypothetical protein		0.45	2.11E-02
SMU.1717c	fused deoxyribonucleotide triphosphate pyrophosphatase/		0.45	3.04E-02
SMU.1662	DNA polymerase III, delta subunit	holB	0.45	6.31E-03
SMU.2024c	50S ribosomal protein L4		0.45	5.26E-03
SMU.1845	transcription termination factor	nusB	0.45	1.01E-05
SMU.929c	hypothetical protein		0.45	9.98E-02
SMU.551	cell division protein FtsA	ftsA	0.45	4.31E-02
SMU.946	putative permease		0.45	7.20E-02
SMU.1416c	putative mutator protein MutT		0.46	1.47E-01
SMU.950	GTPase	engB	0.46	1.49E-05
SMU.842	thiamine biosynthesis protein	thiI	0.46	3.80E-06
SMU.359	elongation factor G		0.46	7.83E-02
SMU.1621c	hypothetical protein		0.46	3.12E-02
SMU.2104a	50S ribosomal protein L32	rpmF	0.46	2.81E-05
SMU.2165	putative SpoJ		0.46	1.68E-05
SMU.855	hypothetical protein		0.46	9.65E-02
SMU.2102	histidyl-tRNA synthetase	hisS	0.46	1.12E-01
SMU.1776c	hypothetical protein		0.46	9.72E-03
SMU.1624	ribosome recycling factor	frr	0.47	8.81E-03
SMU.866	hypothetical protein		0.47	2.38E-05
SMU.1276c	septation ring formation regulator ezrA		0.47	8.79E-03
SMU.1971c	putative thioredoxin H1		0.47	2.61E-04
SMU.84	tRNA pseudouridine synthase A	truA	0.47	2.39E-02
SMU.1109c	putative integral membrane protein; permease		0.47	5.39E-02
SMU.321	hypothetical protein; possible membrane protein		0.47	1.22E-01
SMU.1419	putative transcriptional regulator		0.47	1.34E-01
SMU.1943	putative leucyl-tRNA synthetase	leuS	0.47	1.72E-02
SMU.595	dihydroorotate dehydrogenase 1A	pyrD	0.48	2.74E-02
SMU.229	hypothetical protein		0.48	8.57E-02
SMU.616	hypothetical protein		0.48	1.59E-02
SMU.246	putative glycosyl transferase N-acetylglucosaminyltransferase	rgpG	0.48	1.42E-01
SMU.825	putative RgpAc; glycosyltransferase	rgpA	0.48	1.77E-02
SMU.1297	hypothetical protein		0.48	5.53E-02
SMU.320	putative 5-formyltetrahydrofolate cyclo-ligase		0.48	4.21E-02
SMU.1508c	putative coenzyme PQQ synthesis protein		0.49	4.63E-02
SMU.1824c	transcriptional represor codY		0.49	3.32E-02
SMU.97	CTP synthetase	pyrG	0.49	8.00E-03
SMU.553	hypothetical protein	ylmE	0.49	5.53E-05
SMU.22	putative secreted antigen GbpB/SagA; putative peptidoglycan hydrolase	gbpB	0.49	1.99E-01
SMU.830	RgpFc protein	rgpF	0.49	3.92E-02
SMU.819	putative large conductance mechanosensitive channel	mscL	0.50	6.38E-05
SMU.317	putative tetrahydrodipicolinate succinylase		0.50	1.69E-02
SMU.949	ATP-dependent protease ATPase subunit	clpX	0.50	1.09E-01
SMU.1672	ATP-dependent Clp protease, proteolytic subunit	clpP	0.50	1.12E-01

Table 5. Primers used for qRT-PCR to confirm microarray results.

Locus_tag	Forward primer	Reverse primer
16s RNA	5`-CGCAGGCGGTCAGAAA-3`	5`-TTCCAGAGCACACTATGGTTGAG-3`
SMU.503c	5`-TGCAGCCTGGTTTCATCCTAA-3`	5`-TGGATAGGGATTGGGATGTGA-3`
SMU.984	5`CAACGGGTAATCAGGCAAAAG-3`	5`-TCTACAAAAGCAAGATGACTAGAAGACTCT-3`
SMU.609c	5`-AGAAACACCCCAAGTTGAAGTTG-3`	5`-TTCTTTGGATTTTGCACCTTTGT-3`
SMU.20	5`-TCCCTGATCTAACTGTTTTGTCATCT-3`	5`-TGTCACCCCATCATTTTTTCC-3`
SMU.2146c	5`-TCAGGAGCTTCAGGTCTCTTTCA-3`	5`-CCTTGGGCTTTATAAGCATTGATAG-3`
SMU.1882c	5`-ATTATTCAAAATGGCTGGGTGAA-3`	5`-TTACTAAATCCTCTATTACCTTGAGCTGAGT-3`
SMU.1914c	5`-GGTGCTGGGCAAGGTTATATG-3`	5`-CCGATTCCTCCAGCAATAGC-3`
SMU.625	5`-GCATGAGGGCGTCTATACTTTACC-3`	5`-TTTGCGATCAGCCTTTTCAGA-3`
SMU.910	5`-CAGGCAGCCAACGCATTAA-3`	5`-AGCCCTCGCTCATCATAAGC-3`
SMU.1910c	5`-TTTTCTGTCTACTGGCCGAATG-3`	5`-CAAATGTATAAATGATTACCGTCTTCCT-3`
SMU.1984	5`-TCAGGCTGAGCTTTATGAGCTTAA-3`	5`-CCTTGTATGAATTTGCCTGTTCTTG-3`
SMU.1896	5`-AGACATGTTAGCCGCTGTTGAAG-3`	5`-AAGCGCCTGTTCCAATCGTA-3`
SMU.1841	5`-ATTGGCCCTGGTGATGTTAACT-3`	5`-CCACTGGCAGCAATCTTTTTC-3`
SMU.1865	5`-TTAAAGGGCATTGGTCCCTACA-3`	5`-CGTGCTATAACGCGCATAACA-3`
SMU.1915	5`-TTGGATGTCTATGCGAATGCA-3`	5`-ATGGTTTTCCCCAGATCAAAGA-3`
SMU.932	5`-GCTGGTCTTGAAGGGATTTATCTC-3`	5`-TGCCTCAATAATGGTCAAATCACT-3`
SMU.882	5`-CGGAAGCCATGACCTTAGCT-3`	5`-AGGCGTTCCTACCTGTTCCA-3`
SMU.1961c	5`-CCCTTTGACAACAGCTTGCA-3`	5`-CAACAGCATTGACTGCCATTG-3`
SMU.1837	5`-AATGGTATTTATGCTGCGCAAA-3`	5`-CCGCCTCGCAAGATAACGT-3`
SMU.574c	5`-TGCTATGGTTGCAGGCATTATC-3`	5`-AATCTCCGCCGGGTTTATAAG-3`
SMU.12	5`-CAGCAAATGTTATGGAAGCACTTT-3`	5`-CACGTGAAATGCGTGTATTATCAA-3`
SMU.1339	5`-CGGCGACA CAACAGCACTTA-3`	5`-CGTCACGATCCAAAGCATAGC-3`
SMU.424	5`-CCAGTCAGCGTCAAGGAAGAA-3`	5`-GACACAAATGCGCGAGAAAA-3`
SMU.44	5`-CAAGAGTCTGAGCGTGTGATGAA-3`	5`-TCTATAGGCTTCGCTACCATCTGA-3`
SMU.440	5`-CAACGCCTTTTGGCAATGT-3`	5`-CGTATCGCTGTCTGTCAAAGAAA-3`
SMU.672	5`-CGTGAGTATGCTGCCGAACTT-3`	5`-CAGGCTTGAGCAGAATTTGTTG-3`
SMU.764	5`-CAGATGGTATTGGTCGTGATGCT-3`	5`-CAGCTCCCTCTTTCCATTTGG-3`
SMU.504	5`-GGACTGGAGGAAAAAGGCAAT-3`	5`-GAATAGAGCTCCACCACCAATAAAA-3`
SMU.840c	5`-CAAGAGTCTGAGCGTGTGATGAA-3`	5`-TCTATAGGCTTCGCTACCATCTGA-3`
SMU.182	5`-TGAAAAAACAAGATTGGCAATGTG-3`	5`-AAATTGCCCCCCTGAAAGTT-3`
SMU.1474c	5`-CAGCCAGCTAAGGCGAGAAA-3`	5`-TGACGCTGCGTTCCTTCAC-3`
SMU.770c	5`-GAGGTCAATCAGAGCGTTCGT-3`	5`-ATTTCCCGGATCCATATAACCA-3`
SMU.1496	5`-TATGGTGCGGGCAGCTTTAT-3`	5`-TTATTATGGCCGCGTGTCATAT-3`
SMU.496	5`-ACTTGGCATCTCCGCTTCTG-3`	5`-AGGTGCAATAGCCAAGACTTTTTT-3`
SMU.1515	5`-TTGGATGTCTATGCGAATGCA-3`	5`-ATGGTTTTCCCCAGATCAAAGA-3`
SMU.629	5`-CGCTAATGCGGCTCTTGAA-3`	5`-ACGAATATCCGCTGGAATTTGT-3`
SMU.1764c	5`-TCGTATTGCTAGACCGAAGTCAGA-3`	5`-GCCATAATGCTCCAATTTTGTTT-3`
SMU.127	5`-AATGCAGCGTATCCGTGATG-3`	5`-AAGCCGCCTCTTCTCCTACTG-3`
SMU.1735	5`-CCGTATTGACCGACCCTACAA-3`	5`-TCCAGCGAAGCCAATTAAGG-3`
SMU.1117c	5`-TGCGCGACTGTTTATGACAAC-3`	5`-CCTGCTGCATTATGACCAGCTA-3`
SMU.575c	5`-ACCGATTCCAACCACCGTTA-3`	5`-AACCCAAGGAATCAACCCATT-3`
SMU.173	5`-TTAACTTGGATCCCAAACAAGGA-3`	5`-GCTAATTGGCGCAAAAATACCA-3`
SMU.956	5`-ATACCACGCTTGCTAAGAAAGGA-3`	5`-TGGACGAACACCCATTGCTT-3`
SMU.80	5`-CATGAGCCCATTGGGTCTAAA-3`	5`-GTGCGCCTTCTCTAAGAGTCCTA-3`
SMU.1976c	5`-AAAAATCACAGCAGGCAATCG-3`	5`-AAATCCCGCTGCAAAATCTG-3`
SMU.2028	5`-GCGAACGGCGACTTACTCTT-3`	5`-AGGTGCCCAGGTTGAATTCTT-3`
SMU.1532	5`-AGGAAGCCGGTCGTCTAAAAG-3`	5`-TCTGCCACATCTGCCTTAACG-3`
SMU.870	5`-CTAGTGCTTCGACAGCTGCTGTA-3`	5`-TTATTTGGCCGCATGATGAA-3`
SMU.1276	5`-AAAATGCAACACTGACAGAGCAA-3`	5`-AAGAGCTTAAGGGCAATATCAAAGC-3`
SMU.22	5`-CTAGTGCTTCGACAGCTGCTGTA-3`	5`-CTACCCAAGGACCTAATGATTTAAC-3`